











# BACTERIA IN RELATION TO PLANT DISEASES

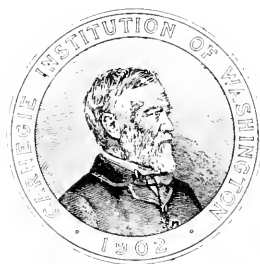
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VOLUME TWO

HISTORY, GENERAL CONSIDERATIONS, VASCULAR DISEASES



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## ERRATA.

Page 8 line 20 read *only*.  
       " 33 " 1 " *Metcalf*.  
       " 56 " 4 " 1897.  
 Plate 3 last line read *See plate 4*

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## INTRODUCTION.

This volume really begins the subject of bacterial diseases of plants, the first volume having had for its aim only the clearing of the ground by a discussion of methods of work and the general subject of bacteriology. Whatever in that volume relates to specific diseases of plants was introduced merely by way of illustration, or to provoke interest in what should follow.

The first part of this second volume deals with general questions relative to bacterial diseases of plants; the history of the subject, the distribution of bacteria on the surface of plants, the questions involved in the terms parasitism and symbiosis, the action of the bacteria on various tissues, the reactions of the plant, the interrelations of animal and plant parasites, and, finally, the problems relating to prevention. The wilt of cucurbits, the black rot of crucifers, and the yellow disease of hyacinths are then dealt with in separate chapters.

In researches of this kind, covering as they do a relatively new and rapidly enlarging branch of science, the point of view changes with great frequency. To-day the interest will be centered on one phase of the subject, to-morrow, perhaps, on some quite different aspect. Fortunate for the experimenter if the new aspect do not require entrance into unfamiliar fields of discouraging complexity. For anyone to cover adequately by the experimental method a whole branch of science if it be a large one, is manifestly impossible. There will always be portions slurred over. The best that one can do is that which I have tried to do, *viz.*, to point out at frequent intervals gaps in our knowledge, to express things clearly and honestly, to distinguish between verified fact and speculation, and, finally, to leave each subject in somewhat better shape than I found it.

The following pages are based in great part on data obtained as the result of a multitude of experiments made by the writer and his assistants, but all sources of published information have been considered.

During the writing of this monograph it has often happened that the ink on some chapters would scarcely be dry before the results obtained from new experiments would require some part of it to be rewritten. In this way during the last ten or fifteen years the subject has been worked over and over, some chapters being rewritten a dozen times, in whole or great part. This, while greatly increasing the scientific value of the work, has certainly not tended to improve its style.

The long period covered by these experiments must also serve to explain why the description of particular organisms does not in all respects follow the recommendations of the chart recently issued by the Society of American Bacteriologists, many of these studies having been completed before that was begun. To make them all conform would delay

this publication indefinitely since the writer no longer has living cultures of some of these organisms.

Troubles of other sorts have been encountered frequently and the reader would be surprised, no doubt, to know how difficult it has been to obtain exact information on some of these subjects.

Some definite rule must govern the citation of literature when it is very abundant as in case of some of these diseases, *e. g.*, potato rots and pear-blight. Purely agricultural or horticultural literature has not been cited in this monograph unless it has a direct bearing on questions under consideration. Many speculative writings have been excluded. For this reason literature on any given disease earlier than that definitely ascribing it to a bacterial origin is, as a rule, not cited. Any other rule would have led to an endless number of citations of very little worth. Perhaps not enough exclusions have been made. In this matter it has been thought best to err on the side of fullness. Probably also some papers have been overlooked since the literature is scattered through many languages. Occasionally a citation has been made simply to show geographical distribution.

As mentioned in vol. I the writer considers it advisable to state whenever possible the exact temperatures at which experiments were made, but it happened frequently in the great mass of notes from which the following pages have been compiled, that the expression "room-temperature" is used. It may therefore be useful to certain readers to know that the Washington room-temperatures, *i. e.*, those of our laboratory, vary roughly as follows: Summer temperature 25° to 35° C., occasionally 38° to 40° C.; winter temperatures (heated rooms) 18° C. to 27° C., usually about 25° C.; spring and autumn approximately 20° to 25° C.

While not averse to synthesis, the writer has usually followed the analytical method. In general, "lumping" things not known positively to belong together is a worse proclivity in natural history than excessive subdivision. Further experiments are often necessary, and until such time it is best to keep separate subjects not demonstrated to be identical; at least the writer has striven to follow this rule. The whole trend of modern scientific research is toward analysis of phenomena, and only in the later stages of knowledge do combinations come in properly to round out a subject thoroughly worked over.

When one has to deal with many diseases some sort of nosology becomes necessary. That which appears to be most convenient for the purposes of this treatise is, first of all, the simple subdivision into three large groups: (1) the vascular diseases; (2) the parenchyma diseases without hyperplasia; and (3) cankers, tubercles, and tumors in which there is a more or less distinct hyperplasia. The reader should remember, however, that classifications are only conveniences.

There is marked bacterial occlusion of the vessels in those diseases which I have classed as *vascular*—occlusions so extensive as to render this feature of the disease most conspicuous, but it does not follow that there is not also some destruction of the parenchyma. Vascular bundles are not on the surface of the plant, and some preliminary bacterial destruction of the surrounding parenchyma must always occur before the disease can take on its true vascular character, except perhaps in those comparatively rare cases where the inoculation happens to be made directly into some bundle. Moreover, in later stages of these vascular diseases bacterial pockets of greater or less extent are often formed in the parenchyma, especially in its softer parts. The extent to which these closed cavities occur varies greatly in different diseases. In the brown-rot of potato and tomato they are numerous and often fuse into large tracts of disintegrated tissues (fig. 1). In Stewart's disease of sweet-corn, on the contrary, they are neither very large nor very numerous. Exception, however, should be made of the inner husks where they are common.

In the remaining groups of diseases, occurring with or without hyperplasia, it is not uncommon to find occlusion of some of the vessels, although the first and principal disturbance occurs in the parenchyma. As examples of this may be cited the basal stem-rot of



Fig. 1.\*

\*FIG. 1.—Cross-section of potato stem of plant 13 (1895) inoculated with *Bact. solanacearum* by needle-pricks May 27, and fixed in strong alcohol June 11. Outer tissues are very little affected except on one side (top of drawing) where there is much disorganization. At X there is a deposit stained red by safranin. This local red stain occurs here and there in other parts. Twelve crystal-sand cells occur in the outer phloem. Stem hollow. Actual diameter of section in longer axis, 4.5 mm. Slide 194 (2).

potato, the spot disease of beans, the black-spot of plums, and the olive-tubercle. Bacteria frequently occur in the vascular bundles of plants suffering from these diseases especially after considerable destruction of the parenchyma, but they are not vascular diseases in any such specific sense as the wilt of cucurbits or the black rot of cabbage. Occasionally also tumefactions, or the stimulation to growth of dormant organs, may be induced by organisms which appear to have little in common with those which regularly produce tubercles and tumors, *e. g.*, the normal action of *Bact. solanacearum* on the tomato, inducing the premature development of adventitious roots and the occasional swelling of tissues when inoculated with cultures which have lost their virulence. The true tumors, *i. e.*, the crown-galls, appear to be an exception to this rule. In these I have not seen any bacterial occupation of the vessels or intercellular spaces. Various subdivisions of these three groups, especially of the second and third, will become necessary and will be made use of in the proper place.

With these explanations and qualifications, we may proceed to the subject in hand, noting, in conclusion, that in this volume as in the preceding the illustrations, so far as possible, have been drawn from the writer's own material and were made under his personal supervision, mostly by James F. Brewer. In case of drawings from sections the writer not only selected the part to be illustrated but also checked-up the finished drawing line by line under the microscope, so that a fair degree of accuracy may be assumed. In most instances, however, the slide number is given under the figure, and, in case of doubt, these slides are on file for reference in the collections of the Department of Agriculture.



## HISTORY.

### THE EARLIEST WORKERS.

The earliest workers in any field of science deserve special consideration. They are like pioneers in a new country, and are usually poorly equipped for their task. If, under such circumstances, a man makes substantial additions to human knowledge, he deserves corresponding credit. Such a man is what we might call a born investigator. He is able not only to see his problem as a whole, but also to see it in its parts, and to determine their interrelations.

The earliest investigators in the field of plant-diseases due to bacteria were Burrill, Prillieux, Wakker, Comes. These men wrought independently—the first in the United States, the second in France, the third in the Netherlands, and the fourth in Italy. All are yet living. To these names I would add that of Woronin, whose one contribution was the discovery of bacteria in the root-nodules of the Leguminosae.

Burrill's principal contribution consists in the discovery of the bacterial origin of pear-blight. The disease had been known in the United States for a hundred years and at times had been very destructive. A multitude of hypotheses had been propounded to explain the mysterious phenomenon, none of which really explained. Into this obscurity and confusion Burrill let a flood of light by addresses and papers published between the years 1878 and 1883. So far as this country is concerned, he may be said to have won over the public in 1881. Many things yet remained to do—things afterwards done by Arthur and Waite—but on the main proposition, namely, that pear-blight could be attributed only to bacteria, Burrill's experiments appeared to be conclusive. Burrill's work was done at the University of Illinois, located at Urbana, where he holds the chair of Botany. Burrill subsequently published short papers on several other bacterial diseases, *i. e.*, potato-rot, disease of maize, and disease of broom-corn. But to none of these subjects does he seem to have been able to give his undivided attention, most of the conclusions depending in part at least upon the work of students. He likewise published papers on fungi, notably the Uredineae of Illinois, in connection with Seymour.

Prillieux published his first paper on a bacterial disease of plants in 1879. This consisted of an account of a microscopic examination of wheat-kernels in which he found clouds of a micrococcus-like organism eroding the interior into distinct cavities. This is generally known in literature as the rose-red disease, or Micrococcus disease, of wheat-kernels. Prillieux did not make any pure cultures or inoculations, and the disease seems to be a rather uncommon one, so that no one has been able in recent years to control his observations, but the account of his microscopic examination is explicit and his figures are not obscure. Subsequently, Prillieux published on various other diseases of plants ascribed to bacteria, *e. g.*, on the olive-tubercle and the Aleppo pine knot, but most of his energies have been given to the elucidation of diseases of a fungous nature. His text-book on diseases of plants is well known. He is professor at L'Institut National Agronomique and was formerly in charge of the plant pathological laboratory, No. 11 Rue Alesia in Paris.

Wakker published his first paper on the yellow disease of hyacinths in 1883. Subsequently he published four other papers on this disease, the last in 1889. For a long time his statements, mostly in Dutch, were overlooked or not generally accepted as conclusive, but none of the early work was better done, and numerous experiments made by the writer have shown that he was entirely right in his main contentions. The yellow disease of hyacinths is a genuine bacterial disease and can be induced in the bulb by inoculating the

leaves with a yellow organism, the disease progressing in just that slow regular way described by Wakker as a result of his own inoculations. Wakker worked at that time in the laboratory of Hugo de Vries in Amsterdam, under a royal grant obtained by the hyacinth growers of Haarlem. Afterwards, for five years, he was in charge of one of the Javan sugar experiment stations. He has published various papers on fungous and other diseases of plants, those of recent years being devoted largely to the sugar-cane.

Comes was one of the earliest workers in this field. He recognized bacteria in the tissues of various diseased plants in southern Italy as early as 1880, and published a number of papers on *Bacterium gummi*, which he believed to be a widely distributed parasite attacking many plants. He did not, however, grow the organism properly in pure cultures and secure infections, nor describe it so that one can now be certain of its identity. He early turned his attention to other subjects, much of his energy being given to teaching botany in the Royal Agricultural College at Portici, of which he is now director. He is the author of a general text-book on botany, of a book on diseases of plants, and of elaborate treatises on tobacco, as well as of numerous minor publications, and his students are scattered all over Italy. He seems never to have had any doubt as to the occurrence of bacterial diseases in plants.

Sorauer also being one of the voluminous writers on plant pathology should be mentioned here, because, as early as 1886, he saw clearly that Wakker and the others were right. Sorauer regarded the bacterial diseases of plants only from the general standpoint of a writer and student of plant pathology. He examined various such diseases microscopically but did not make pure cultures or inoculations therefrom, not having had the necessary grounding in bacteriological technique. He endured much obloquy in his earlier years for steadily maintaining the existence of such diseases in opposition to Hartig and his school, but he has lived to see his contentions established. Sorauer is the author, among other works, of the most elaborate and important German handbook on diseases of plants, the third edition in three volumes having been completed recently. He is also the founder and editor of the *Zeitschrift für Pflanzenkrankheiten*.

Savastano and Arthur began work a little later than the others when there were not so many difficulties in the way.

Savastano conceived the idea that the olive-tubercle was caused by bacteria. He demonstrated the constant occurrence of bacteria in the knots, isolated them therefrom in culture-media, and produced typical overgrowths on healthy olive shoots by punctures into which minute quantities of the culture were inserted. Cavara went over Savastano's experiments soon afterward with similar results, and the writer and his assistants have done the same thing in recent years (see vol. I, plate 2). This was Savastano's most important incursion into bacteriology. He described, however, in an imperfect and fragmentary way a number of other diseases as bacterial, most of which are really such. He is the author of a handbook, *Patologia Arborea Applicata*, and the editor of the *Bollettino della Arboricoltura italiana*. Savastano held for many years the chair of forestry in the Royal Agricultural College at Portici and is now director of the experiment station at Acireale in Sicily.

Arthur repeated and verified Burrill's work on pear-blight and carried the investigations somewhat further, demonstrating that infectious fluids filtered through porous clay cups lost their power to produce the disease, whereas the residue containing the bacteria was as infectious as ever. Arthur began his experiments about the time that Burrill ceased, and continued them for a number of years, publishing a half dozen papers on pear-blight and the organism to which it is due, these papers forming his most important contribution to plant bacteriology. He was at that time stationed at the Geneva experiment station in central New York. Since then he has been professor of botany in Purdue University, Indiana, and has published interesting papers on fungi and other plants. He is the author, with Barnes and Coulter, of *A Handbook of Plant Dissection* and has published with

MacDougal a collection of essays entitled *Living Plants and their Properties*. His cross-inoculations of Uredineae in recent years have settled many doubtful points. For many years he was one of the editors of the *Botanical Gazette*.

Earlier than any of these writings is the paper by Woronin (1866) announcing the discovery of bacteria in the root-tubercles of lupins. His conclusions were based simply on microscopic examinations, but they were confirmed by Beyerinck two decades later (1888) and since that date a great literature has arisen. Woronin studied under De Bary and afterwards published several papers in conjunction with him. He was born in St. Petersburg and lived there during the latter part of his life. He is justly famous for his beautifully executed monographs on various pathogenic fungi: *Tubercinia*, *Sclerotinia*, etc. Beyond this one early research, so far as known to the writer, he did nothing with bacteria.

About the same time (1868) the Frenchman, Davaine, a man of marked originality and excellent powers of observation, showed that certain bacteria were able to produce a soft-rot in plants when inoculated, and this observation was subsequently confirmed by van Tieghem and many others (see *Destruction of Cell-walls*, etc.).

In conclusion, one ought not to forget Anton De Bary (1831 to 1888). The present has its roots in the past and his profound influence still lives. De Bary published nothing on bacterial diseases of plants but he made it possible for others to do so. Directly or indirectly all the early workers in this field were influenced by him. The same is true of those who followed, and all of us are much indebted to him.

#### THE GENERAL ATTITUDE OF PATHOLOGISTS AND BACTERIOLOGISTS.

In the consideration of this topic it is hardly worth while to dip into the ill-digested mixture of fact and speculation published prior to 1874. I shall therefore begin with Sorauer.

In the first edition of his comprehensive *Handbook of Plant Diseases*, published in 1874, Sorauer makes no mention of bacterial diseases of plants. A great many diseases are included, but none of this type.

De Jubainville and Vesque writing in 1878 mention a "cellular rot" of potatoes, radishes, carrots, and beets, occurring in the soil or in cellars, but they attribute it to soil ill-adapted to the plants or to improper cultivation. No mention is made of bacteria either as the cause of this rot or of any other disease mentioned by them.

There is nothing on this subject in Winter's little book, published in 1878.

Reinke and Berthold, who published in 1879, found that potato-rot could go on independently of the presence of fungi. They say: "But there are also wet-rotten potatoes which show no trace of these *Pyrenomyces*." A little farther on we are told correctly that: "A tuber can become wet-rotten without ever having been diseased by *Phytophthora*." And again,

It has already been mentioned above that in addition to the *Myxomycetes* occasionally found upon wet-rotten potatoes, bacteria are also present. In fact, these bacteria are a constant accompaniment of the wet-rot, and this is an indication as to the true cause of this decay. If a potato-tuber infected by *Phytophthora* lies in the wet, it passes quickly over into the wet-rotten condition. As soon, however, as the first symptoms of the wet-rot appear, bacteria are found in quantity in the wet-rotten tissue. Notwithstanding this one might think that the *Phytophthora* caused the wet-rotten decomposition, \* \* \* and that the bacteria hastened the decay only secondarily. But if one inoculates a tuber, which is entirely sound and free from *Phytophthora*, with the bacterial fluid from a wet-rotten potato, there is always a local production of the wet-rot in the tuber which has been kept moist, and not rarely does it become totally wet-rotten in a very short time. The cause of the wet-rot can be considered to be, therefore, only the bacteria and the ferments produced by them, the tissue of a potato-tuber being only specially predisposed for the action of the bacteria by the *Phytophthora*.

These men repeatedly produced wet-rot of the potato by direct inoculations. Their method consisted of cutting out a little tetrahedron from a tuber, to a depth of 10 to 15 mm. A drop of fluid containing the bacteria was put into this wound. The tetrahedral piece of the tuber was then put back and pressed in. When the tuber was placed with the wounded side up, the cut portion usually dried out, a cork-layer formed under the cut surface, and the wet-rot did not occur. On the contrary, when the tuber was placed with the wounded side down against wet paper under a bell-jar, in a saturated atmosphere, there was always more or less decay of the tuber, and sometimes within 2 or 3 days the whole interior became wet-rotten. In no case did Reinke and Berthold experiment with pure cultures nor could they have had any active parasite, but we are warranted in believing that sometimes at least their mixtures of bacteria were free from filamentous fungi. They found different tubers to possess very different powers of resistance, as will be mentioned in another place.

The first edition of Frank's *Diseases of Plants*, published in 1880, contains a brief chapter on root-tubercles of Leguminosae, but nothing on bacterial diseases of plants.

The following translation from page 27 of Hartig's *Lehrbuch*, published in 1882, shows the general attitude of botanists and pathologists at the time Burrill and Wakker were working out their interesting and beautiful results.

With the pathological processes in plants they have nothing whatever to do; in fact, I have never on any occasion found the schizomycetes in the interior of a closed plant tissue, and they have nothing to do with the falsely so-called rotting processes of dead plant tissue. Of course, this does not exclude them from a share in the destruction of dead vegetable substance whenever they find an easy access to it. Evidently the interior of the plant is difficult of access to them because the open circulatory paths are wanting, which in animals make possible their rapid distribution with the blood. Also the circumstance that the wall of the plant-cell is nitrogen-free and is mostly very thick in comparison with the size of the schizomycetes, must be an obstacle to the wandering of the schizomycete from one cell to another. Finally, also, the formation of humus acids in the dead plant tissue will tend to prevent the multiplication of the schizomycetes.

In Worthington G. Smith's book (1884) there is nothing on bacteria as a cause of disease in plants, although two long chapters are devoted to "The Potato Disease," as though there were but one.

In 1884, in his *Comparative Morphology*, etc., De Bary considered the subject very briefly, but much more cautiously than Hartig:

As Hartig has already pointed out, bacteria living in plants parasitically have scarcely been observed. The generally acid reaction of plant parts may be a partial explanation of this. Recently, however, Wakker has described as the yellow sickness, a disease of hyacinths in Holland, in which the characteristic symptom consists in the presence of slimy yellow bacterial masses in the vessels, etc. \* \* \* More exact investigations upon this phenomenon must be awaited.

The following year, in his *Vorlesungen*, De Bary devoted two pages to this subject. Parasitic bacteria as causes of plant disease have been only infrequently observed. The most of such diseases are due to animals and plants of other groups, especially to fungi. Wakker's yellow disease of hyacinths is described briefly from that author's first papers with the remark that "successful infection experiments and the exact following out of the life history of the bacterium are still to be awaited." In the same way, Burrill's work on pear-blight and apple-blight is mentioned, without other comment than that "in Europe this phenomenon, so far as I know, is not known, or at least has not been carefully investigated."\* Prillieux's studies of changes in wheat-grains are mentioned with the remark respecting the micrococcus that "its importance as a cause of disease can not be judged with any certainty from the short account. It may turn out to be only a saprophyte appearing in consequence of other injuries." Finally, De Bary mentions the wet-rot of potatoes, studied by Reinke

\*The disease of the peach tree, of the Lombardy poplar, and of the American aspen, mentioned by De Bary on Burrill's authority as bacterial diseases are now believed to be due to other causes.

and Berthold, with the remark that recent experiments by van Tieghem on potato-tubers, bean-seeds, cactus-stems, etc., seem to confirm these results.

Otherwise expressed, these facts may be stated as follows: As a rule, saprophytic bacteria may, *under special conditions*, attack, make sick, and destroy living plant tissues as facultative parasites.

In the third edition of Zopf's Spaltpilze, published in 1885, bacterial diseases of plants are discussed as follows:

Much rarer [than in men and animals] are the cases in which we can speak of the genuine parasitic action of schizomycetes in plant-organs. The best-known example is the familiar disease of potato-tubers known as "wet-rot" caused by the butyric acid ferment (*Clostridium butyricum*), through which the tissues of the potato are entirely destroyed and converted into a vile smelling fluid-mass (Reinke and Berthold). It remains to be seen whether the phenomenon known in Holland as "the yellow disease of hyacinths," and recently described by Wakker, belongs here strictly speaking. Its characteristics are the appearance of enormous numbers of yellow schizomycetous colonies in the vessels and (at blossoming time) also in the intercellular spaces and cells of the parenchyma.

Perhaps the rarity of schizomycetous diseases of plants lies in the generally acid reaction of the juices of plants, perhaps also in their lower temperature as compared with the animal body, and finally the formation of protective cork is perhaps also to be considered (p. 3).

In the second edition of De Bary's Vorlesungen (1887) Arthur is said to have confirmed and extended Burrill's work on pear-blight.

In the second edition of his Handbook, published in 1886, Sorauer devotes 38 pages to diseases due to bacteria, namely, to rot of the potato, white rot of hyacinth bulbs, rot of onion bulbs, Comes's gummosis of the tomato, Prillieux's rose-red wheat grains, and various stem and leaf reddening. These pages deal with field appearances, the results of microscopic examinations, and to a limited extent with what I have designated in vol. I as *direct* infection experiments. Sorauer accepts the doctrine of bacterial disease of plants without reserve and says: "Beyond doubt, in course of time, a large number of rot diseases will be recognized." Of exact bacteriological methods there are no suggestions in this book. Dr. Sorauer's own observations appear to have been limited to microscopic examinations and a repetition of such crude infections as were made by Davaine, Hallier, Reinke and Berthold, van Tieghem, and others. The bacteriosis which he had in mind is that which occurs when tubers and bulbs are exposed to excessive moisture, with a restricted supply of oxygen. From his own observations and experiments on potatoes, he could say with reasonable assurance that "the wet-rot or rot may be produced artificially without the aid of *Phytophthora* by inoculating bacteria into sound tubers. The decomposition phenomena of the two diseases are essentially different." He had also observed that the bacteria could penetrate through the open lenticels into the tubers.

The statements in Sorauer's book on Die Schaden der Einheimischen Kulturpflanzen (1888) are essentially the same as in his Handbook, only much more condensed. His general standpoint is expressed in the following sentence: "The bacteria are certainly much more dangerous to living plants than has hitherto been recognized."

De Toni and Trevisan in vol. VIII of Saccardo's Sylloge Fungorum (1889) gave short descriptions of all the species of Schizomycetes known to literature.

Under bacillus "Sectio 6 species endophytobiae, destruentes," the following species are included: *Bacillus vuillemini* Trev., *B. oleae* (Arcang.) Trev., *B. ampelopsorae* Trev., *B. radicicola* Beyerinck, *B. hyacinthi* (Wakk.) Trev., *B. hyacinthi septicus* Heinz, *B. sorghi* Kell., *B. amylovorus* (Burr.) Trev.

Laurent, writing in 1889, has the following:

Many fungi which invade the higher plants have the property (propriété) of perforating the cell membranes by the intervention without doubt of a special *zymase*. The germs of the ordinary bacteria could easily penetrate into the leaves by way of the stomata when they are brought there by the wind or other agents. But having reached the stomatic chamber, they would have to traverse the cell membranes or to insinuate themselves between the cells. In order that this last mode of

infection might succeed, it would be necessary to suppose bacteria mobile, capable of crawling into the intercellular spaces, something quite improbable (ce que est assez peu vraisemblable), or else filamentous forms with continuous development, in the manner of the mycelial filaments of fungi. As to short non-motile bacteria, they would have to traverse the cell membranes. \* \* \* Now I have determined that the cellulose, even of the most tender varieties, resists perfectly, exposed to the air, a great number of kinds of common bacteria. The solvent action of *Bacillus amylobacter* takes place only in the absence of oxygen. Nevertheless, according to Vignal, the *Bacillus mesentericus vulgaris* secretes a yamase which digests the most tender celluloses. I have made the same observation in case of a *Bacillus subtilis*, which, when developed in mycoderma on the surface of a liquid, separated the cells of a bit of potato situated in the depths of the same liquid. In conclusion, if the penetration of the cell-membranes of plants is not a general property of the bacteria it does occur and may perhaps be developed in a particularly favorable medium.

This condition is nevertheless not sufficient to enable the bacteria which are outside to establish themselves in the tissues of plants. It is necessary also to take account of the resistance peculiar to living cells, a resistance the mechanism of which is still entirely enigmatic. Among animals, the pathogenic bacteria overcome this difficulty by the production of substances more or less toxic, rapidly diffused through the entire organism by way of the blood stream. The higher plants have the advantage of being much more resistant to the movement of the microbes and of their secretions through their tissues. Consequently there exist few bacterial diseases among plants, while in the animal kingdom there are a great many of them.

Kirchner (1890) mentions two bacteria, *Bact. termo* Ehr. which has been said to take part in the destruction of cells in the interior of sorghum-stems, and *Clostridium butyricum* Prazm., which "causes the wet-rot and dry-rot of potato-tubers and the rot of onions; also on the roots of apple trees, pear trees, plum trees, and cherry trees."

Scribner (1890), says in his preface: "We are told that bacteria cause pear-blight and some other plant diseases," but does not mention the subject in the body of the text.

In his *Diseases of Plants*, published in 1890, Marshall Ward does not mention bacteria as one of the causes, although he also has a chapter on "the potato disease."

Comes (1891), like Sorauer, admits without reserve the existence of such a class of diseases and treats the subject constructively, in a space of 38 pages. The organisms considered are *Micrococcus amylovorus*, *Bact. gummiis*, *Bact. zcae*, *Streptococcus bombycis*, *Bacillus sorghi*, *B. amylobacter*, *B. hyacinthi*, *B. caulicorus*, *B. vuillemini*, *B. olcae*, *B. ampelopsorae*, and *B. radicola*.

Ludwig (1892) also recognizes the existence of bacterial diseases of plants and devotes about 8 pages to the subject, but this does not include any original work.

Loverdo (1892) recognizes the existence of bacterial diseases and devotes a number of pages to an account of the sorghum blight attributed to *Bacillus sorghi*.

By far the best paper of its time (1892) is that written by Migula for the Middle Java Experiment Station. Without personal knowledge of bacterial diseases, but with a knowledge of most of the literature, and a logical mind, Dr. Migula applies the ordinary rules of pathological inquiry to the question of the existence of bacterial diseases of plants, and comes to the conclusion that five only out of about twenty which are mentioned deserve to be considered as clearly established. These are pear-blight or apple-blight, sorghum-blight, Burrill's bacterial disease of maize, Heinz's rot of hyacinth, and Kramer's wet-rot of the potato.

Russell, who published the same year as Migula, also admits the existence of bacterial diseases of plants, but like Migula, his observations were based mainly on the work of others. In tables at the end of his paper, he mentions 13 diseases as of established bacterial origin and 9 as "probably of bacterial origin," *i. e.* 22 in all. The following were included in his first class: pear-blight, Burrill's sorghum-blight, Burrill's corn-blight, Wakker's yellows of hyacinth, Heinz's hyacinth rot, tuberculosis of olive, and of Aleppo pine, blight of oats, Arthur's carnation blight, Bolley's potato scab, Burrill's wet-rot of potato, Kramer's wet-rot of potato, sugar-beet disease of Arthur and Golden. In the second class were geranium-blight of Prillieux and Delacroix, Halsted's cucumber and tomato-blight, Halsted's root-rot

of vegetables, Garman's cabbage-rot, Sereh disease of sugar-cane, Comes's gum diseases, Halsted's celery-blight, Ludwig's white slime-flow, Ludwig's brown slime-flow.

Bacteria are not recognized as the cause of any grape diseases by Viala in the second edition of his *Diseases of the Vine*, published in 1887. In the third edition of this book, published in 1893, two French diseases of the grape are said to be due to bacteria: *Pourriture des grappes* and *maladie du coup de pousse*.

In 1894, in the English translation of Hartig's *Diseases of Trees*, edited by H. Marshall Ward, it is stated that "Only in extremely isolated cases has it been placed beyond doubt that these low organisms are the primary cause of disease in plants." Wakker's disease of hyacinth, the wet-rot of potatoes, the tubercle of Aleppo pine and of the olive tree, and the blight of the pear are more or less grudgingly admitted to be diseases of this class.

Concerning the first, it is said that "under normal conditions the bacteria do not attack perfectly healthy bulbs" and that "a species of *Hyphomyces* almost always accompanies the disease." Under wet-rot of the potato the editor hazards the following statement:

It is extremely probable that in this and other similar cases the minute bacteria travel in the tissues down the tubes of the filaments (hyphae) of the fungus, feeding on the decomposing protoplasmic contents of the latter.

Concerning pear-blight we have the following:

Lately a disease of apple and pear trees has been described by J. Burrill, of Urbana, Ill., under the name of "blight," the cause of which, according to this investigator, is to be ascribed to the invasion of a bacterium. The disease appears to bear resemblance to the tree-canker produced by *Nectria ditissima*; and as, in the case of this fungus, large numbers of small gonidia resembling bacteria are produced in the cortex, it remains to be seen whether this disease has not been erroneously ascribed to a bacterium.

Prillieux in his text-book on *Diseases of Plants*, published in 1895, devotes 37 pages to bacteria. He admits the following as diseases of bacterial origin: Rose-red disease of wheat grains; wet-rot of potatoes; white-rot of hyacinths; potato scab; gangrene of potato stems; disease of grape bunches in France; Mosaic disease of tobacco; blight of mulberries; point-rot of tomato fruits; black spots in potato tubers; spot disease of sorghum; yellow disease of hyacinths; gummosis of the vine; pear-blight; olive tubercle; pine tumors; white slime-flow of trees; brown slime-flow of trees; black slime-flow of trees. In general, Prillieux's book gives the impression of one who works rapidly and only with the microscope.

Von Tubeuf in his book on *Plant Diseases*, published in 1895, devotes 10 pages out of 611 to this class of diseases. In the introduction he says:

While only a few diseases of men and warm-blooded animals are due to the true fungi, etc. \* \* \* the infectious diseases of plants are caused almost exclusively by fungi. \* \* \* Even the few bacterial diseases thus far described are almost all still incompletely investigated and abundantly doubtful in two directions. In case of some we have unquestionably to do with a plant disease, more or less exactly known, and it is only the cause of the same which is not yet fully investigated. In these cases, the question then arises whether the disease is due to a microorganism at all and whether this is or is not really a bacterium. But in other cases it is doubtful whether the phenomena, in connection with which the appearance of the bacteria has been observed, are truly to be considered as diseases. On this account we will speak with reserve and briefly upon the bacterial diseases, a labor essentially lightened by the bringing together of the bacterial diseases in the *Lehrbuch der niederen Kryptogamen* of Professor Ludwig, 1892, and the critical examination of the same from the bacteriological standpoint, by Dr. W. Migula.

Then follows a brief account of the 5 diseases reckoned by Migula as of bacterial origin, and also the mention of 17 others ascribed to bacteria by various persons, and concerning which Dr. von Tubeuf says in the introduction, speaking very cautiously, as one unfamiliar with the subject:

But we will also here refer briefly to those diseases in which bacteria are suspected of being the cause.

Hallier (1895) asserts the existence of bacterial rots but does not attempt to make a list of them. They follow fungi or act independently, but in all cases, Hallier would have us believe that the bacteria themselves have developed out of the plastids (protoplasmic granules) of the fungi.

The second edition of Frank's book on plant diseases, published in 1896, contains 1,213 pages (3 volumes), 13 only of which are devoted to bacterial diseases of plants. There is a good deal of internal evidence (careless proof-reading, etc.) going to show that this book was thrown together very hastily. The arguments in the chapter on bacteria in particular are vague and inconclusive. This is made sufficiently plain by the following paragraph:

On the contrary, in the plant-world the bacteria have a very subordinate place in the production of diseases. The striking bacterial action on the plant is consequently not of a pathological character, but a profitable symbiosis, to wit, that in the root-tubercles of the Leguminosae. Where one has perhaps the right to speak, in connection with plant diseases, of bacteria as causes of disease is in a number of rot phenomena of certain underground plant parts. Sorauer proposes, under the hypothetical assumption that these diseases are caused by bacteria, to designate the same by the common name *rot* or *bacteriosis*. But, in truth, we have here to do, for the most part, with very ordinary rot phenomena which represent the regular end stage of other diseases, in which, demonstrably, the genuine higher fungi, or also other external factors, are the true primary disease-producer, and decay-bacteria appear only secondarily in the tissue, dead in consequence of the disease, and, powerfully hasten the progress of the destruction of the diseased plant parts on account of the decay which they set up; not rarely, also, are associated with other decay-loving fungi, especially moulds. But because, in isolated cases, it has been possible to produce similar decay phenomena by inoculating sound plant parts with bacteria taken from rotting plants, a number of pathologists insist on viewing these bacteria also as primary causes of disease. Moreover, some cases of hypertrophy, that is of true gall formation, are known in which bacteria are said to be the cause. In the following pages we register all that is known of an authoritative character. From this it will be seen that a satisfactory proof for the acceptance of pathogenic bacteria has not been furnished, and that many times people have sought to help out with a supposition of bacteria as a cause, in diseases which may be brought about through another cause, or the cause of which is not easy to discover, or which also have not been sufficiently investigated by the observer in question.

Then follows a discussion of the wet-rot of potato, in which there is no mention of the then most important paper on the subject, *viz.*, Kramer's; the white and yellow rot of the hyacinth, in which the two diseases are confused, in which Wakker's five papers are condensed into four lines, and in which there is no mention of Heinz's paper. The most of the two pages on this disease is devoted to Sorauer and the digest concludes with the statement that "there is at least yet no proof of a pathogenic bacterial action." The rot of onions is discussed briefly from data published by Sorauer. Bolley's work on potato scab and beet scab is then considered, following which are notes on various other diseases: olive-tubercle, Aleppo pine gall, rose-red disease of wheat grains, pear-blight, etc. Under bacteriosis of the sugar-beet there is no mention of Kramer's paper or of Sorauer's second note published in 1892.

There is evidence throughout that many of the original papers were never seen or, if seen, were not read.

The last edition of Flüge's large general work on microorganisms (1896) contains 1,385 pages, of which three and one-half are devoted to bacterial diseases of plants, following Migula and Ludwig (vol. I, p. 418; vol. II, pp. 308 and 328).

Most general treatises on bacteriology do not discuss this subject at all and even so extensive an annual compendium of bacteriology as Baumgarten's *Jahresbericht* omits all mention of bacterial diseases of plants, although the title is inclusive.

In the first volume of his *System* (1897) Migula devotes 12 pages out of 376 to this subject, going over the literature in the same careful way as in his earlier publication. Out of 29 diseases mentioned, the 8 following are considered to be of proved bacterial origin: Sorghum-blight, pear-blight, Cobb's gum disease of sugar-cane, olive-tubercle, Kramer's wet-rot of potato, Heinz's rot of hyacinths, Arthur and Bolley's spot disease of carnation, and Smith's wilt of cucurbits.



The English edition of von Tubeuf (1897) does not differ essentially from the German.

Frank's Kampfbuch, published in 1897, is chiefly interesting in this connection, because in it the author announces his changed views respecting the existence of bacterial diseases of plants. Concerning them we have the following very cautious recantation (p. 201):

Whether bacteria can be the cause of disease in plants is always a question to be considered with circumspection. In case of the potato-rot this doubt was formerly so much the more justified because we had learned to know a genuine thread-fungus, the *Phytophthora*, as the cause of this disease, and consequently the suspicion at once arose that perhaps this fungus was really the true cause of the disease and might have paved the way for the entrance into the potato of the decay bacteria. I myself have held fast to this doubt until quite recently, but must give it up as a result of my own investigations recently instituted.

In his Vorlesungen, published in 1897, Fischer takes the ground that there are no bacterial diseases of plants and can not be any for reasons cited, to wit, the bacteria can not enter the plant except through wounds, and their development in the latter is soon stopped by the formation under them of an excluding layer of cork. Stomatal infection is altogether impossible for the reasons stated:

Die *unverletzte Pflanze* steht mit der Aussenwelt nur durch die Spaltöffnungen in offener Verbindung, die selbst sich darauf beschränkt, dass das gegen die Zellen ganz abgeschlossene System der luftgefüllten Interzellularräume mit der Aussenluft kommuniziert. Wenn durch den Wind oder durch Regen Bakterienkeime in die Spaltöffnungen geführt werden, so gelangen sie von hier aus nur in diese Interzellularräume, wo ihnen ausser dampfgesättigter Luft nichts weiter geboten wird, wo alle Nährstoffe fehlen, ohne die keine Bakterienspore auskeimt, keine Bakterienzelle sich vermehrt.  
\* \* \* Alle diese Fähigkeiten fehlen den Bakterien, gegen die eine unverletzte Pflanze vollkommen geschützt ist. Aber auch die *verwundete Pflanze* würde nur in den geöffneten, verletzten Zellen Nährstoffe für Bakterien darbieten, eine Quelle, die bald dadurch abgeschnitten wird, dass unter der Wundfläche eine undurchlässige Korkschicht (Wundkork) entsteht, die jeden weiteren Säfteaustritt aus der Wunde verhindert. Die Wunde bleibt nicht feucht, die verletzten Zellen schrumpfen und trocknen ein und damit ist den Bakterien der Eingang genau so versperrt, wie an der unverletzten Pflanze. Ihr drohen demnach auch keine Wundinfektionskrankheiten durch Bakterien, deren Weiterver schleppung in der Pflanze gleichfalls unmöglich ist.

The following is a translation of the entire paragraph:

Exclusive of the tubercle bacteria whose wonderful relation to the Leguminosae has been described already (Vorl. X), no single example is yet known of bacteria which can insinuate themselves into the closed living cells of a plant. The *uninjured plant* stands in open connection with the outer world only through the stomata, which connection is so limited that the system of air filled intercellular spaces connects with the outer world but is entirely closed to the cells. When bacterial germs are forced into the stomata by wind or rain, they here reach only into these intercellular spaces where nothing further is offered to them than vapor-saturated air, where all nutrient substances are wanting, without which no bacterial spore can germinate, no bacterial cell can multiply. Even when such bacteria as can dissolve cellulose (the methane bacteria) are brought into the intercellular spaces they can not nourish themselves here, and can not develop their peculiarity of dissolving the cell-wall. Consequently only those parasitic organisms can penetrate into the plant with results, whose spores have brought along with them sufficient nutrient substance so that they can germinate in pure water, so that they can overcome the lack of nutrient substances which they meet with at first, and can open their attack on the protective cell-wall at their own expense. This requirement is fulfilled by the spores of parasitic fungi, which with their reserve stuff push out a germ-tube, which now bores directly through the epidermis of the plant (potato fungus, *Phytophthora infestans*) or which first penetrates into the intercellular system through a stoma (rust fungi), and from here boring through the cell-wall, multiplies in the cells, or at least sends into them special side branches of its mycelium as sucking organs (haustoria). All these peculiarities are wanting in the bacteria, against which an uninjured plant is fully protected. But also the *wounded plant* offers food for bacteria only in the opened, injured cells, a source which is soon removed by the formation under the wounded surface of an impenetrable cork layer (wound cork) which entirely prevents any further flow of sap from the wound. The wound does not remain moist, the injured cells shrivel and dry out, and consequently the entrance of the bacteria is exactly so barred out as in the uninjured plant. Consequently, there is not the least danger of wound-

infections by bacteria, whose further progress in the plant is also impossible. Moreover, the result of an injection into the living plant of bacteria, even those pathogenic to animals and men, is easily predicted: There is no development in the intercellular spaces, or in extensive wound surfaces there is a wholly insignificant and soon extinguished multiplication. The experiments have exactly so concluded and need no further mention. Nevertheless new descriptions of plant diseases caused by bacteria keep springing up, and, truly, what worthless descriptions and what non-critical experiments. That in diseased plants bacteria are often found in great numbers is certain, but they have here always settled down only saprophytically (Metatroph) upon tissues broken down and destroyed by genuine fungi, and now, of course, help in the further work of destruction, and may also lend to the further progress of the disease a special aspect. But exclusive of other injuries, such as frost, animals, etc., the first attack upon the plant is brought about by fungi, not only in sickenings of uninjured plants, but also in case of wound-infections, which often are greatly extended by fungi and are converted into incurable injuries. From the bacillary gummosis of the grape vine to the scab of the potato, all so-called bacterial diseases of plants are of other origin, the bacteria being only saprophytic contaminations (metatrophe Verunreinigungen), not self-conquering parasites (pp. 131, 2).

Wehmer's views, propounded in 1898, are not essentially different from the earlier views of Frank, or those of Alfred Fischer. They are sufficiently indicated by the following translation from his long paper on potato diseases:

Thorough investigations of the bacterial rot are not so far to be found in literature. The few contributions which exist take up at random several specific cases and explain the problem with especial regard only to the bacteria, the tuber as a living organism being very little considered. The conclusions reached as to the "pathogenic" characters of the bacteria are indeed generally accepted to-day, but are not yet really sufficiently well grounded. Moreover, they are not pertinent, as I shall endeavor to show. \* \* \* For us, in this connection, the first sort of decay (primary rot) is of interest practically to the exclusion of the other, and will be somewhat fully considered in various directions. The question arises here especially whether we actually have to do in these cases with Schizomycetes capable of attacking living sound tissue. This supposition, all things considered, is to be definitely denied: *There is manifestly no bacterial sickening of sound tubers*; consequently in a literal sense also no "primary" rot—this is *always* secondary. In proof of which I have gathered together a pretty comprehensive mass of experiments.

Wehmer also regrets that Frank should have abandoned his former safe position to accept the doctrine that bacteria can be independent causes of disease in plants.

That other factors are truly primary and that the rot with its bacteria is only secondary has been already pointed out by Frank in opposition to earlier statements (Pflanzenkrankheiten, 2 Aufl. Bd. II, 1896, p. 22). For leaving this standpoint there was really from first to last no reason; on the contrary its soundness was rather to be more exactly established by experiment. \* \* \*

Bacterial decay is only the last stage of the injury begun by environment, and even where it apparently attacks sound uninjured tubers one can demonstrate without difficulty that such is not the case. But of course it is easier from the simple discovery of the bacteria in decayed tissue to infer the pathogenic action of these organisms; in this way without trouble the numerous plant bacterial diseases, such as fill the literature of the day, are established.

Smith (1899) criticised Fischer's statements and maintained the existence of bacterial diseases of plants, citing numerous experiments by various people in proof of his contention.

Fischer (1899) answered Smith, maintaining that no one would doubt his having gone over the literature quite carefully; that all of the statements he had thus far examined rested, manifestly, on inexact observations or worse; that, for a book of the scope of his Vorlesungen, his statements were entirely sufficient; and, finally, that "there has not yet been published a single proof for bacterial plant diseases which meets all the requirements of exact bacteriology."

Smith then made reply (1899 and 1901) to Fischer's criticisms, defending himself and other investigators, the validity of whose statements had been called in question, illustrating three bacterial diseases by means of numerous heliotypes from photomicrographs. Since 1901 no one has ventured to question their existence.

Peglion (1899) describes briefly the following as bacterial diseases: spot of hemp stems, mal nero of the vine, tubercle of the vine, tubercle of the olive, and blight of the mulberry.

Nadson's Russian paper, published in 1899, is a popular account drawn from the literature of the subject. He admits the existence of plant diseases due to bacteria.

In his Text-book of Plant Diseases, published in 1899, Massee devotes  $4\frac{1}{2}$  pages out of 470 to bacterial diseases, mentioning bacteriosis of tomatoes, hyacinth bacteriosis, pink bacteriosis of wheat, black-rot of cabbage, olive tuberculosis, and the brown-rot of tomato, eggplant, and potato. The author's perfectly safe standpoint is expressed in the following words: "At the present day numerous plant diseases are attributed to bacteria, some truly, others doubtfully so."

Out of 1,350 odd species considered in the second volume of Migula's System (1900), 30 are at present of more or less interest to the plant pathologist. The action of the remainder, when introduced into living plants, is nil or unknown, mostly unknown. This book, like the Sylloge Schizomycetum of De Toni and Trevisan in Saccardo's Sylloge Fungorum, is devoted to a description of species rather than to a consideration of their pathogenicity.

The last views of Hartig, which did not differ very materially from those held by him 20 years earlier, are sufficiently illustrated by the following quotations from his Lehrbuch, published in 1900:

In fact, bacteria have been found thus far only in the tissue of those plants the cells of which are of a parenchymatic nature or are very thin-walled, as in bulbous and tuberous plants.

The yellow disease of hyacinths is erroneously ascribed to onions and is further discussed as follows:

The bacteria do not attack sound well-ripened bulbs under normal conditions. Some sort of wounding is necessary, such as readily occurs during the lifting of the bulbs and their storage in another place, or else the bulbs are already attacked by fungi, among which a *Hypomyces* in particular is an almost constant accompaniment of the rot disease. In a damp situation the bacteria force their way into the wound and cause its decay.

Bacteriosis of the potato is dismissed with 6 lines upon "wet-rot of the tubers." Pear-blight is discussed in 4 lines; sorghum-blight is mentioned in 6 lines.

Of the olive tubercle it is said:

In the olive forests gall-formations from the size of a pea to that of a walnut often occur in enormous numbers. *These galls soon die and show in the crevices [of the dead galls!] large bacterial masses* (figs. 203 and 204). *But whether these are the cause of the gall-formations is not yet proved.* [The italics are mine.]

No other diseases are mentioned and this chapter closes with the following paragraph:

Recently still other diseases have been ascribed to bacteria, without, however, furnishing the convincing proof by means of infection experiments that the Schizomycetes are the cause of the diseases. With these belong also the slime-flows of trees.

In December 1900, Erwin F. Smith gave a lantern-slide lecture in Baltimore, Maryland, before a joint session of the Society for Plant Morphology and Physiology, and the Society of American Bacteriologists, illustrating fully three bacterial diseases from original photographs and photomicrographs in his possession (Science, February 15, 1901).

Weiss (1901) mentions, as of bacterial origin, wet-rot of the potato, white or yellow rot of hyacinth bulbs, beet-tip rot, and scab of potatoes.

Less important bacterial diseases are probably—rot of onions, rose-red wheat kernels, \* \* \* and the mosaic disease of tobacco.

This author's standpoint is expressed in the following introductory remarks:

The plant diseases due to Schizomycetes are of *subordinate importance*, while the bacterial diseases of animals and men are of the *greatest importance*. The bacterial diseases of the cultivated

plants in general presuppose *previous disease* due to fungi. They bear the name of *rot diseases* or *bacterioses*. Through the transfer of bacteria from diseased to sound plants the sickening of sound plants can be induced.

In 1901, in his *Disease in Plants*, under "Exudations and Rotting," Marshall Ward, then leading English writer on plant pathology, has the following on bacterial diseases:

In many of these cases bacteria abound in the putrefying mass, and some evidence exists for connecting these microbes causally with the disease in a few of the more thoroughly investigated cases, but in no case has this been sufficiently demonstrated; and considering the ease with which bacteria gain access *via* wounds caused by insects and fungi, as well as by other agents, the necessity for gain proof must be insisted upon before we can accept such alleged examples of *Bacteriosis*. \* \* \* Wet-rot of potatoes may be due to various fungi, and, in excess of water, to putrefactive bacteria. \* \* \* The principal agent in the destruction of the tissues is *Clostridium*, an anaerobic bacillus which consumes the cell-walls but leaves the starch intact. \* \* \* The rotting of bulbs, roots, etc., has been much discussed during the last few years in the pages of the *Gardeners' Chronicle*, *Zeitschrift für Pflanzekuh.*, and elsewhere. The principal references to Bacteriosis—the rot in which bacteria are stated to be the primary agent causing these and similar diseases—may be found in Massee, *Diseases of Plants*, pp. 338–342, and more fully in Russell, *Bacteria in their Relation to Vegetable Tissue*, Baltimore, 1892; and in Migula, *Kritische Uebersicht derjenigen Pflanzenkrankheiten, welche angeblich durch Bakterien verursacht werden*, Semarang, 1892.

The most convincing accounts, however, are since that date; see Smith, *Pseudomonas campestris*, *Cent. f. Bakt.*, B. III, 1897, p. 284, and Arthur and Bolley, *Bacteriosis of Carnations*, Purdue University Agr. Expt. Station, 1896, vol. VII, p. 17. Woods has lately shown that this disease is due to Aphides only, the bacteria having nothing to do with the disease primarily, *Sligmonose*, *Bull.* 19, U. S. Dept. Agr., 1900: but it is necessary to bear in mind that actual penetration of the cell-walls from without must be proved, as De Bary proved it for germ-tubes of fungi, before the evidence that bacteria are truly parasitic in living plants can be called decisive. This is a difficult matter, but until it is settled we do not know whether these organisms are really parasitic in the sense that *Phytophthora* is, or merely gain access by other means—I have traced them through dead fungus-hyphae—to the vessels, dead cell-walls, etc. The proof of infection *via* water pores and vessels is given for one species by Harding, *Die Schwarze Fäulnis des Kohls*, etc., *Cent. f. Bakt.*, Abt. II., B. VI., 1900, p. 305, with literature. \* \* \*

On *Bacteriosis* in Turnips, see Potter, *Proc. R. S.*, 1901, vol. LXVII., p. 442.

In Conn's *Agricultural Bacteriology*, published in 1901, 5 pages out of 419 are devoted to bacterial diseases among plants, but some of the specific statements will scarcely pass muster, e. g., those respecting the olive-tubercle. The author's standpoint is sufficiently illustrated by the following citation:

It has been claimed that there is no likelihood that bacteria can live under such conditions and that bacterial diseases are, therefore, on *a priori* grounds, improbable or impossible. Even in very recent years this claim has been very vigorously supported, and disputes are still going on in the pages of bacteriological journals, in regard to the question of the existence of bacterial disease in plants. Almost to the very present day, it has been insisted that there is no demonstration that bacteria can produce disease in plants. Although this claim was legitimately urged a few years ago by conservative scientists, it can no longer be held in the light of recent experiments. In the last few years the evidence for such diseases has accumulated rapidly, and to-day the proof of the existence of bacterial plant diseases stands on identically the same basis as the proof of bacterial diseases among animals.

In Neppi's Italian translation of Kirchner's book, published at Turin in 1901, various bacterial diseases are mentioned with the organisms said to be their cause. These are: The red disease of wheat kernels (*M. tritici*), internal rotting of corn-stalks and sorghum (*B. termo*), celery rot (*B. apii*), Bolley's potato scab, Kellerman's sorghum disease (*B. sorghi*), gangrene of potato stems (*B. caulivorus*), brown-rot of potato (*B. solanacearum*), beet disease (*B. betae*), hemp disease (*B. cuboniansus*), wilt of cucumbers, etc. (*B. tracheiphilus*), disease of vine stems (*B. viticorus*), rot of grape bunches, soft rot of potatoes, onions, etc. (*Clostridium butyricum*), tumors on peach branches described by Cavaia.

Plates IX–XIV in Delacroix's *Atlas* (1901) are devoted to bacterial diseases of plants, viz., to tubercle of the olive and of the Aleppo pine, gummosis of vine, red disease of wheat,

yellow disease of beets, grease spot of beans, gangrene of potato, etc. The text is very brief and closely follows Prillieux, except in case of the spot disease of beans, which is based on Delacroix's own work.

In the second revised edition of his large Text Book, issued in 1901, Dr. Sternberg mentions bacterial plant diseases for the first time, devoting 7 pages to the subject, quoting exclusively from the publications of Smith and Waite.

Chester's book (1901) includes descriptions of a few plant parasites, but does not venture any statements as to pathogenesis.

In their large Treatise published in 1902, Miquel and Cambier devote 10 pages out of 1059 to the micro-organisms of plants. They mention 28 species as being of more or less interest in this connection. For general remarks by these authors on the uncertainties hanging over this subject, see citation in the preface to vol. I.

Van Hall's Thesis (1902) maintains the existence of bacterial diseases as proved beyond dispute. He mentions many diseases, having a very good grasp of the literature; and admits the following 15 as of clearly-established bacterial origin: The black vein disease of crucifers due to *Ps. campestris*; the wilt of Solanaceae due to *B. solanacearum*, the wilt of cucurbits due to *Bacillus tracheiphilus*; the yellow disease of hyacinths due to *Ps. hyacinthi*; the bacterial gummosis of sugar-beets due to *B. betae*; the maize disease due to *Ps. stewarti*; pear-blight due to *Bacillus amylovorus*; lilac-blight due to *Ps. syringae*; the olive tubercle due to *B. oleae*; the spot disease of beans due to *Ps. phaseoli*; potato-rot due to various bacteria (*B. solaniperda*, *B. solanacearum*, *B. atrosepticus*, etc.); carrot-rot due to *B. carotovorus*; turnip-rot due to *Ps. destructans*; iris-rot due to *Ps. iridis* and *B. omnivorus*; hyacinth-rot due to *B. hyacinthi septicus*.

The original matter in this thesis will be discussed under the various diseases.

In 1903, in the second edition of his Vorlesungen, Fischer repeats many of the inadmissible statements of his earlier edition, but, nevertheless, gives several pages to a review of a few bacterial disease of plants, dealing briefly with the rot of fleshy roots, potato-rot, the black-rot of cabbage, the mosaic disease of tobacco, and tree-cancers. Concerning the latter we have the following:

Bacteria as the cause of cankers are unknown, for the *Bacillus oleae* which is said to cause the canker-like swellings of the olive-tree is no more legitimized by pathological experiment than many other bacteria described as pathogenic for plants.

It is fitting that these citations should end as they began, with Sorauer's Pflanzenkrankheiten. The second volume of the third edition devotes many pages to the subject of bacterial diseases of plants. This purely didactic review published in 1905, contains the best summary in any general treatise on plant diseases. About 70 bacterial diseases are considered. The statements in it, carefully as the literature has been gone over by Dr. Lindau, show, however, perhaps as clearly as anything, the great need for a re-examination of the whole subject by some one experimentally familiar with it.

Most of the conclusions I have cited in this chapter are to be regarded simply as ex cathedra judgments, or to put it somewhat differently they are to be regarded only as so many evidences respecting the ability of the particular writers to reason logically and arrive at sound conclusions from a maze of contradictory statements. In other words they are literary or legal judgments rather than scientific ones. A good judge must have not only a keen, well-balanced mind, but he must also know the *case* and the *law*. Very few of the writers I have cited appear to have had any extensive acquaintance with this class of diseases, or with the rules of evidence guiding in pathology, and those who have rendered adverse judgments seem to have had none whatever, *i. e.*, they made few observations and no experiments, or only some irrelevant ones. It is no wonder, therefore, that the insight of some of these writers has been much shrewder than that of others, or that most of them should have mingled fact and fancy in nearly equal portions in what they

have published. Nearly all of them have grossly neglected the experimental method. Under special diseases I shall have something to say about particular misconceptions, but, I have not felt called upon to point out all the errors strewn over the pages of these handbooks. Their name is legion and some of them have done service for a generation, having been handed down from one author to another, *e. g.*, the "hyphomycete" that is almost always present in the yellow disease of the hyacinth.

After a consideration of the treatment accorded to bacterial diseases of plants in these handbooks, the reader is well prepared to accept Migula's statement that: "Dieses Gebiet der Bakteriologie gegenwärtig zu den verworrensten und wissenschaftlich am wenigsten durchgearbeiteten gehört" (System Bd. I, p. 312).

In reality, however, the subject is not extraordinarily difficult, if it is approached by the experimental method, not more difficult than new researches in any other branch of science—to cut underbrush and break ground in any field of science is laborious work. This stage is now largely passed and there is considerable definite information on the subject as will appear from what follows.

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## GENERAL CONSIDERATIONS.

### ON THE SUPPOSED NORMAL OCCURRENCE OF BACTERIA IN PLANTS.

We now believe that bacteria do not occur normally in the interior of sound plants. The case is quite different, however, with wounded plants or wilted ones. Frequently saprophytic bacteria have been found in such plants and occasionally mistaken for parasites.

When bacteria are found in the tissues of plants in any great number we may assume that they are disturbing elements, and that if they continue to multiply the result to the host or some portion of it must be some considerable diminution of vitality, even if no specific disease supervenes. Compensations due to symbiosis are not here under consideration.

The former great uncertainty as to the life-history and habitat of bacteria led to many speculations respecting their normal occurrence in the interior of both plants and animals. The belief that they might occur normally in the interior of plants arose from the inexact observations and experiments of various early workers, notably Béchamp and Hallier. The dispute continued for a number of years but was finally settled in the negative.

Béchamp went so far as to maintain that his microzymes were always present in plants and animals, were in fact the simplest components of the tissues and led an independent life after their death and disintegration. Hallier believed that the protoplasmic granules of fungi were converted into bacteria capable of an independent existence.\* Fremy maintained the existence of hemi-organized bodies in the juice of fermentable substances which bodies were converted into yeasts. Trécul believed in similar transformations: granules of organic matter became motile bacteria. In a later time it was still believed by some that bacteria could be cultivated out of the sound interior of plants and animals and were normally present therein, and by others that they arose spontaneously in all sorts of organic substances. That the organic must have developed from the inorganic during some period in the history of the earth seems probable, but we must look elsewhere than to Béchamp and Hallier for evidence.

The amount of ignorance and credulity respecting micro-organisms prevalent in the middle of the last century seems astonishing in the light of our present knowledge. It is, however, the history of all subjects hedged about by difficulties. The beginnings are always foggy.

Pasteur appears to have been the first to show that the sound interior of plants is free from micro-organisms. He experimented on grape-berries, taking some of the juice from the interior under such conditions as to preclude the entrance of surface bacteria and placing it in sterile must which remained sterile, while flasks treated to the washings from the surface of the grapes invariably developed growths of some sort.

In 1879-1880, Chamberland working in Pasteur's laboratory showed that beans taken directly from the interior of their pods were free from bacteria, *i. e.*, did not contaminate culture-media when put into them (see fig. 2).

\*Even in very recent times we have similar views occasionally coming into print, *e. g.*, Dunbar's *Zur Frage der Stellung der Bakterien, Hefen und Schimmelpilze im System* (1907), in which it is maintained that bacteria, yeasts and fungi, are the product of algal cells.

†FIG. 2.—Peas taken from pods less than 18 hours after picking and placed on sterile nutrient gelatin where they have sprouted and grown entirely free from the presence of bacteria. Photographed Oct. 3, 1908, from sample tubes sent the writer by Mrs. A. W. Bitting. Two-thirds natural size.



Fig. 2.†

In 1882, Prof. T. J. Burrill described a *Micrococcus toxicatus* as the essential virus of the Poison Ivy (*Rhus toxicodendron*). It is now believed that the poison of *Rhus* is not due to a micro-organism but to a chemical substance: A non-volatile oil (Pfaff); a glucoside (Syme).

In 1884, Jorisson ascribed the formation of diastase in the higher plants to the presence of bacteria in the tissues. In the following year, however, Laurent pointed out errors in Jorisson's work. Laurent himself reached the conclusion that there are no bacteria normally present in living plants.

Galippe (1887) examined the inner tissues of many kinds of vegetables from the vicinity of Paris and found bacteria so constantly present, except in garlic, that the reader is at once led to suspect some serious error in his methods of work. No quantitative tests were made of the number of bacteria per gram of tissue or per cubic centimeter of juice, and it is possible that those which appeared so regularly in the author's tubes are to be ascribed either to the use of wounded or wilted vegetables, to air contaminations occurring at the time the cultures were made, or to imperfectly sterilized media, especially as his results are not in accord with those of Fernbach.

Galippe first experimented with vegetables grown on a soil supersaturated with sewage-bacteria, *i. e.*, with those grown in the municipal experiment gardens on the plain of Gennevilliers, near Paris.

The vegetables tested were exposed to the Bunsen flame until the surface was carbonized. They were cut with a hot, sterile knife. Each surface of the section was then flamed. Finally, by means of a hot sterile knife (heated above 100° C.) "I detached fragments of the vegetable which were put directly into the culture-fluid, choosing the most central parts. I strove as far as possible to remove sources of error."

His culture-media were: (1) ordinary bouillon; (2) peptonized bouillon with sugar; (3) same, neutralized; (4) saliva peptonized and sugared; (5) same, neutralized; (6) broths from the vegetables experimented upon; (7) same, with peptone and sugar.

A long series of experiments was instituted, the culture-media being inoculated from carrot, onion, celery, parsnip, turnip, potato, beet, lettuce, salsify, leek, cabbage, Brussels sprouts, Jerusalem artichoke, garlic. All gave positive results except garlic. The juice of the latter is said to sterilize culture-fluids.

A second series of experiments was made, using vegetables taken from the market-gardens about Paris. Concerning their origin only one thing was ascertained carefully, namely, they did not come from Gennevilliers. These are styled normal vegetables. The author obtained substantially the same results with these vegetables, *viz.*, the clouding of most of his culture-media.

His general conclusions, therefore, are: (1) the micro-organisms of the soil can penetrate into the tissues of the vegetables with which they are in contact, the mechanism of this penetration remaining to be elucidated; (2) the number of the micro-organisms contained in the vegetables seems to vary with the number in the dung used.

There is no statement in the paper as to how the culture-media was sterilized; where the inoculations were made, *e. g.*, whether in a clean room, free from air-currents; nor as to whether check-tubes of the various culture-media were generally held for comparison and remained sterile. All we are told is that *some* of the many inoculated tubes remained sterile. Concerning these results, as already hinted, conclusions quite different from those of the author might be drawn.

Fernbach carefully repeated the experiments of Galippe and published a paper on the subject in 1888. He found all of Galippe's conclusions erroneous. In all cases, except that of the tomato, pieces of the interior tissue were removed and thrown into the culture-media. In all, 98 different specimens were examined. Of the 555 inoculations only 35, *i. e.*, 6.3 per cent, developed any growths. This number is considered about the minimum of necessary contaminations, arising from air-currents and other imperfect conditions under

which the work was carried on. An examination of the contents of the fertile tubes also confirmed this view.

Fernbach took vegetables just as they came into the market without inquiring where grown, assuming that any soil adapted to vegetables was rich enough in micro-organisms so that they would penetrate into vegetables if this was physiologically possible. He tested potatoes, carrots, turnips, beets, and tomatoes, judging it not worth while to try additional sorts, since experiments on all of the above showed the conclusions of M. Galippe to be erroneous. For culture-media he used neutral veal-bouillon and sugared turnip-water which was very slightly acid. These media were in test-tubes and Pasteur flasks.

The surface of the vegetables was first heated to light carbonization by means of a thermo-cautery. The tomato juice was aspirated out by means of a pipette drawn out at one end and plugged with cotton at the other. The other vegetables were punched with brass cork-borers, cotton-plugged above and sterilized inside of cotton-plugged test-tubes at 165° C.

I obtained with these tubes cylinders of vegetable tissues which I pushed out little by little, and which were sowed immediately by sectioning them with a flamed scalpel. I thus introduced into each tube a volume of tissue varying from 0.5 to 1 cc.

A summary of Fernbach's results is given in the following table:

Vegetable.	Number of vegetables sampled	Number of sowings	Number of sowings which were fertile
Tomatoes.....	26	52	2
Turnips.....	36	199	19
Carrots.....	13	101	4
Beets.....	12	103	10
Potatoes.....	11	100	0
Total .....	98	555	35

We see that there are a certain number of fertile sowings, 6.3 per cent. It could scarcely be otherwise. As a matter of fact, in experiments so delicate there are several sources of error which it is impossible to remove absolutely. The most important is that which arises from germs of the air. These vary greatly in number but are always abundant in a laboratory where there are goings and comings and where one is constantly exposed to currents of air. The practice of filling Pasteur flasks almost daily shows me that, in the laboratory where I have made my experiments (Sorbonne), out of 100 flasks filled there are always 4 or 5 which show growth.

The organisms which developed in 6.3 per cent of the cultures were of various sorts—bacilli, micrococci, molds, etc., in general each tube being occupied by a single sort. A source of error also to be considered is the possible penetration of germs into vegetable tissues as the result of insect depredations, or of injuries due to digging or transportation.

We conclude, therefore, that normal vegetable tissues constitute a perfect filter for microbes and that they can be invaded by them only as a result of causes wholly accidental (p. 570).

In 1888 A. di Vestea repeated Galippe's experiments. This author experimented under much the same conditions as Galippe, *i. e.*, with plants obtained from the market-gardens around Naples and grown on the lowlands where the filth of the city is dumped. He tested especially a variety of lettuce called Roman lettuce. In sampling he made use of a special glass-punch, consisting of a thin tube through which slid a thick-walled tube longer than the first, closed with cotton at the free end, and sliding in a plug of cotton. The whole apparatus was sterilized at 150° C., after the lower half had been introduced for protection into a test-tube plugged with cotton. The plant to be tested was then cut with a flamed knife. The punch was removed from the test-tube and the cutting end of the outer thin-walled tube was then plunged into the cut surface of the vegetable and a piece of tissue removed. The apparatus was then replaced in the sterile test-tube, the tissue was pushed out of the cutting tube by means of the inner tube, which was then used to crush it in the bottom of the test-tube. Finally, some of the liquid which resulted from the crushing was sucked

up into the inner tube and from this transferred to the culture-medium. At the same time sterile bouillon was put on the crushed tissues, thus giving two series of cultures. The culture-medium was sterilized veal bouillon. Numerous experiments led di Vestea to the following conclusions:

(1) Cultures made from plants which he gathered himself or which were brought to the laboratory (Lab. de la Clinique Cantani, Naples) fresh from the country remained regularly sterile whether in vacuo or exposed to the air.

(2) If the same vegetables were left exposed to the air for a day or more, a new sample very often gave positive results.

(3) Finally, whenever the author worked with vegetables *brought in from the market* he always obtained fertile cultures.

"This last result is to be explained, I believe," says di Vestea, "especially by the fact that the gardeners and produce dealers water their vegetables, to keep them fresh, with water which is usually swarming with bacteria."

In 1888, Dr. Bernheim claimed to find bacteria in the interior of seeds of cereals, but Lehmann, in whose laboratory he had worked, discredited his views, stating that Bernheim's studies were very incomplete researches made under direction and published during a vacation period without his teacher's knowledge or consent.

The same year in discussing Bernheim's paper Dr. Buchner says that he (Buchner) obtained negative results from his tests of normal vegetable tissue for the presence of bacteria.

In 1889, Dr. Lehmann, discussing Bernheim's work said: "Normal plant seeds are germ-free." In 43 gelatin-roll-cultures made out of at least 800 fragments from the interior of beans, chestnuts, maize, peas, and almonds, he obtained only 6 bacterial colonies, which undoubtedly came from the air. At the conclusion of his paper in the Archiv für Hygiene he says: "I summarize the second part of my work thus: That Buchner certainly is right in considering Dr. Bernheim's pellicles for non-living formations—but that in opposition to Buchner's view they did not consist of fat, but of salts."

Laurent experimenting on several occasions with various plants, *viz.*, seeds of barley, maize, and lupin, tubers of potato, bulbs of onions, roots of carrot and chicory, and the fleshy tissues of Cereus, Agave, and Carica, obtained the same results as Fernbach. The interior of these plant parts was found to be free from bacteria.

In 1890, Laurent showed that the sap flowing from the cut surface of healthy vine-stems contains no bacteria, *i. e.*, none of these organisms were taken up by the roots. Eleven tests were made on as many young potted grape-vines, placed for a few weeks in winter in a hothouse. In each case the shoot was flamed, cut, the cut end reflamed, and then plunged into a tube plugged with cotton and containing 10 cc. of sterile veal broth. In 24 hours, 5 to 10 cc. of sap had oozed out and mingled with the culture-fluid. The branches were then removed and the tubes incubated at 30° C. In a week's time only 1 tube developed bacteria. Some of the fluids were neutral, others were slightly acid, and the rest were slightly alkaline.

In 1891, Kramer found no bacteria in the interior of sound potato-tubers (for a long review in English see American Naturalist, 1897, pp. 123-138).

In 1892, Russell reached the same conclusion respecting several plants.

In more recent years Hiltner has reached the same conclusion respecting the interior of sound seeds. He found the tissues of those he tested always free from bacteria.

The writer has sometimes found bacteria in fleshy roots supposed to be normal, and the surface of which had been properly sterilized, but these had been dug for some time. The parenchyma of healthy plants is always or almost always free from bacteria. Probably the vascular system, especially of some parts of the roots, frequently contains bacteria, and certainly they must be present to some extent in those plants in which tyloses are abundant, if the latter are due to the stimulus of bacterial products as believed by the writer (see fig. 30 and page 91).

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## BACTERIA ON THE SURFACE OF PLANTS.

### OBSTACLES TO THEIR ENTRANCE INTO PLANTS—OBSTACLES TO THEIR MULTIPLICATION IN PLANTS.

The young vegetative parts of plants are covered by the *epidermis*, a skin of close-set cells interrupted here and there by stomata, but not easily permeable to water. This epidermis when unbroken offers great resistance to the entrance of harmful micro-organisms.

Its surface is often reinforced by *cutin* a still more resistant layer which is sometimes developed to a very marked degree. Some plants also turn aside water and whatever that may contain, by a waxy bloom, *e. g.*, the cabbage. A dense layer of soft hairs may have the same function, as on the surface of a peach fruit or the stem of a composite. These devices render it difficult to wet the actual epidermis lying under the cutin, wax, or lanugo.

In older parts the epidermis is displaced by *cork* a many-layered, close-celled, very impervious, very indestructible covering which keeps out fluids and also keeps them in so perfectly that the special kind found on the cork-oak is used by civilized man everywhere for this very purpose. Its use to the plant is obvious. Only through wounds or through certain natural openings, known as *lenticels*, can bacteria pass this very perfect barrier (see fig. 3).

The plant then is naturally very well protected against bacteria, except as I will point out in a following chapter.

The surface of plants, as we shall see a little later, is often covered by a variety of bacteria and some of these are likely to find their way into the tissues whenever they are wounded, but if they do gain an entrance either through wounds or through some natural opening, they can in the vast majority of cases take no advantage of it because they are saprophytes, *i. e.*, they are not adapted to the conditions present in the plant. And even if they happen to be parasitically inclined they are often debarred from further progress by the fact that the wounded plant does

not contain enough water for their needs. In such cases they make either no growth or such a very slow growth that the plant has time to erect a physical barrier to further progress in the form of a cork-layer cutting out the affected tissues from the body of the plant. This happens very frequently in potato-tubers attacked by various soft-rots. It

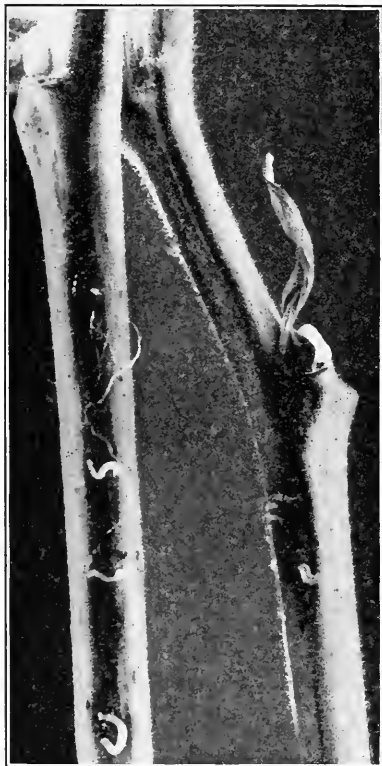


Fig. 3.\*

\*FIG. 3.—Young shoots of mulberry inoculated with *Bact. mori*, showing cirri of bacterial slime oozing to surface through lenticels. Inoculated by needle-pricks Jan. 4, 1909. Photographed (enlarged) Jan. 9. The dark stripe is a sunken diseased area.

occurs often also in leaf-spot diseases. It is not always a perfect protection, however, since in some weak portion the parasite may break through the barrier and form a new center of infection. This I have observed many times.

The chemical obstacles are equally interesting, although our knowledge of many of them is far from exact. There can be no doubt, however, as to their existence. There are probably few substances in plants which bacteria can not be educated to tolerate in test-tube cultures, especially when the inoculations are very copious and the doses of the antiseptic are small at first. The conditions in nature, however, are somewhat different. Especially are the number of bacteria accidentally introduced into the plant undoubtedly, in most cases, vastly fewer than we introduce on our needles or with the hypodermic syringe.

It is a common laboratory experience that culture-media which will not cloud when inoculated with minute doses of bacteria will do so when inoculated with larger quantities of the same bacteria.

I found the acid parenchyma juice of cucumbers exerted a decidedly retarding influence on *Bacillus tracheiphilus* and the same was true of hyacinth juice on *Bact. hyacinthi*; with *Bact. stewarti* I could not get any growth in a very acid tomato-juice. In one instance a slight reduction in the acidity of a potato juice by the use of sodium hydrate enabled an organism to grow readily—a very slight reduction in proportion to the total acid present. Many bacteria are quite sensitive to the organic acids occurring in plants, *e. g.*, malic acid, citric acid, tartaric acid, and there can be little doubt that these exert a protective influence. I have never found any bacterium that would grow in pieplant juice\* or in orange juice. Galippe states that he could not obtain any growth in garlic juice. I made one trial with the same result.

Whole families of plants contain bitter or aromatic substances, and some of these must undoubtedly be regarded as protective substances, indeed, some of them as salicin, methyl salicylate, thymol, menthol, camphor, cinnamon oil, mustard oil, are distinctly antiseptic. Other plants contain alkaloids, glucosides, etc., which may be assumed to be more or less protective.

Tannin is a substance very widely distributed in plants; we do not know its functions very well, but, as Tschirch has suggested with reference to germinating seeds, one of them may be that of antiseptis. It might inhibit bacterial development either directly or by oxidation into more active colorless or brown compounds. In this connection see interesting observations by Hiltner on a germicidal or inhibiting substance extruded by sprouting seeds of legumes (p. 124). Anthocyan is thought by some to be antiseptic.

Appel has pointed out that potato-tubers, the flesh of which turns a reddish brown on cutting (oxidation of tannin compounds), are much more resistant to *Bacillus phytophthorus* than those which remain white. The latter were rotted easily on inoculation, the former either not at all, or very slowly. In an experiment by the writer made in test-tubes using this same organism, growth was certainly more rapid in potato-juice steamed at once and consequently remaining pale, than in a portion of the same juice that was allowed to oxidize 24 hours before steaming. Dr. O. Loew states that he found bacteria absent or rare in brown, curing tobacco, and the writer confirmed microscopically some of his results. In the samples shown to me bacteria were certainly not abundant.

The list of weak to moderately strong antiseptics derived from plants is a long one, as every bacteriologist knows. We are accustomed to look on them, for the most part, with little favor because they do not accomplish all we desire, *i. e.*, they are not actively germicidal, but the requirements of the plants are undoubtedly less, by far, than our own, and in a particular case the antiseptic substance may be all sufficient for the plant, *i. e.*,

\*In August 1908, what appeared to be a bacterial soft rot of pieplant (base of the petiole) was received from Nashville, Tenn. A fungus, somewhat resembling a *Pythium*, was associated with the bacteria.

enough to stop the beginnings of mischief, or to delay growth until cork-barriers can be formed.

There may be also unknown substances in the plant, enzymic or other, having a special protective function. If there are not some such substances, especially in the roots, it is difficult to understand how plants live at all, since the roots are broken and wounded by many sorts of animals, and grow in a substratum swarming with micro-organisms. The writer's thought comes back frequently to the question: What protects the roots? And in the case of water-plants, we may add, the stems also?—for their surface is constantly bathed by water containing innumerable bacteria. Wiesner has classed all plants into ombrophobic and ombrophylic: the one sort having many devices for keeping out excessive moisture, and rotting readily when wetted unduly; while the other sort wets readily and resists decay. He found roots of all plants extraordinarily resistant to decay. Probably the spongy nature of many roots and of stems of aquatics saves them from decay, *i. e.*, they are too dry for the bacteria to obtain a foothold.

#### THE EPIPHYTIC SPECIES.

This brings us naturally to the question of what bacterial organisms are likely to be found on the surface of plants. Animal pathologists know that the skin harbors a variety of bacteria, and surgeons have devised a very elaborate technique for surface sterilization.

The surface of a plant, while not excreting fermentable substances to the extent of the animal skin, has, to some extent, its own peculiar flora, and a similar technique is necessary if one wishes to make sterile wounds.

The swarming bacterial flora of the surface layers of the earth (the soil proper), the multitude of organisms known to occur in all waters that have touched any fertile portion of the earth's surface, and the incalculably great number contained in the animal waste used in agriculture, expose the surface of the plant, particularly in agricultural fields and hothouses, to all sorts of contaminations. Sporiferous and non-sporiferous forms occurring naturally in the soil, in water, or in dung are very likely, therefore, to be found on the surface of plants and sometimes to such an extent on vegetables, fruits, and salads as to make their consumption in a raw state the beginning of various intestinal disturbances.

Some of the dung-bacteria found on plants are green fluorescent on culture media and grow so rapidly that they swamp all slow-growing forms. These break up nitrogen compounds into simpler substances, often into free nitrogen. One meets them very frequently on the surface of plants grown in hothouses or in heavily manured fields.

The accidental surface organisms are not the most interesting, however, nor apparently are they the most frequent. There is a great deal yet to be learned about the subject, but we know enough already to assert that the surface of plants harbors habitual residents, bacterial epiphytes, so to speak. Those with very generalized or very limited needs are found on a great variety of plants, while others seem to be much more restricted even if they are not confined to particular plants or groups of plants. The subject is perhaps one of no great practical interest, but none the less very interesting from the standpoint of pure science.

Many so-called soil-bacteria, water-bacteria, and sewage-bacteria are not such *per se*, these particular organisms having their true home on the surface of plants. *Bacillus cloacae* was described from sewage, but its true habitat is undoubtedly the surface or decaying parts of plants. *Bacillus coli* was described from the intestinal tract where it is usually, if not always, very abundant, but according to Metcalf, Prescott, and others, it occurs on the surface of grain much as though at home there. Barber's temperature curve for this organism makes me think, however, that it is quite as well adapted to the animal body. Recently John R. Johnston, working in my laboratory, has found it to be the cause of the bud-rot of the coconut-palm.



When we know better the bacterial flora of the surface of plants we shall be able to classify the soil, water, and sewage organisms more satisfactorily, for undoubtedly a part of these belong specifically to soil, to water, to sewage, that being their natural habitat.

The organisms peculiar to the surface of plants must be assumed to remain dormant under unfavorable conditions, often probably for weeks or months coming into activity whenever dewfall or rainfall renders a little food available, which will be whenever any dead tissues, or extruded soluble substances, even the least, are present, and that is always.\* The kind of organism, which grows in this moistened dead tissue or in extracts of it, will depend, of course, on what is offered since the requirements of different groups of bacteria are as various as the composition of different plants. This, at least, is what a variety of observations would lead me to believe.

It is a fact well known since Cohn's time that beans thrown into water will give almost invariably a green fluorescent culture. Why? The simplest explanation is that their composition favors the presence of this sort of organism on their surface. Housewives have long known that canned fruits usually keep, while canned green corn almost invariably spoils. Professional canners have learned the same thing, and consequently employ the autoclave. They know that peaches are easily canned while green corn often spoils in spite of special precautions. Why? The answer is that the surface flora of the one is quite different from that of the other, corn favoring the growth of some very resistant spore-bearing bacteria, while the fruit does not. The surface of potato-tubers also affords a home for certain spore-bearing bacteria very resistant to heat. One was found to be so common that Robert Koch called it the "potato bacillus," a name that has continued in use and been extended to other similar forms. It is reasonable to suppose that they are not accidentally present on the tuber—but rather quite at home there from the fact that they convert potato-starch very readily into soluble reducing substances which they can use as food. The retting of flax is due to bacterial organisms which are probably common on the surface of the plant, and perhaps peculiar to it. Almost any sample of hay cut up and boiled for a few minutes in water, to destroy the nonsporiferous forms, will yield *Bacillus subtilis*, or what passes for that.

These are old and well-known illustrations. There are many others, less familiar, and the subject is still so new that I can barely touch its surface. The writer ran into it in 1893 when he first attempted to make cultures of *Bacillus tracheiphilus* from cucumber-stems, and like most tyros did not realize the necessity for entering the plant through a sterile surface. Invariably I dragged rapid-growing surface organisms across my cut surfaces and these contaminated the plates and either crowded out the slow-growing parasite or were mistaken for it. I learned my lesson finally. Continually since then I have had to do with surface-organisms in one way or another, mostly in the way of devices to circumvent them, and often in literature I have had occasion to observe how they have led unsuspecting persons astray.

It is the rule in all diseases due to bacteria, whether of plants or animals, that the parasite is followed sooner or later, often somewhat closely, by saprophytes of various sorts, the kind depending, of course, on what organisms are peculiar to the surface of particular plants or animals. Given the universal presence of these surface organisms able, in the absence of restraining influences, to use starch, sugars, acids, asparagin, etc., as food, and it is easy to see how any little mass of dead or dying tissue, if sufficiently moist, might be invaded and occupied by them speedily, this being their way of life. It is also easy to understand, if surface sterilization has been insufficient, how one can get the wrong organism using what seem to be proper methods, especially if the right thing is a rather slow grower

\*Düggeli found certain saprophytic surface bacteria extremely abundant (usually millions to billions per gram) in drops of fluid exuded from the water-pores of seedlings of *Triticum spelta*. The amount of solid matter available for their use in this fluid he determined to be only 0.05 to 0.1 per cent, of which about half was ash. The most common form in this fluid was *Bacterium fluorescens*.

while the saprophytes are rapid growers. The latter are particularly perplexing and deceptive when through weakness or death of parts they rapidly invade the deeper tissues where the student expects to find only the parasite. If, also, there is some antagonism between the two, then the surface organism may gain complete ascendancy in particular portions of the tissue and the cause of the disease may disappear altogether, or appear so sparingly on the plate-cultures that it is entirely overlooked. Literature bristles with examples, the olive-tubercle being a good recent case. Yellow and white non-pathogenic bacteria occur very frequently in olive-knots and on plate-cultures these come up sooner than the parasite. I learned this long ago and Petri, in Rome, has confirmed it recently. Ignorance of this fact led one of the Italian workers to spend two years in carefully working out the biology of the wrong organism—a potato bacillus.

An experiment station bulletin on the bacterial spot of the carnation was based wholly on the wrong organism, a common surface-growing yellow form. The true parasite is a white organism making a quite different type of spot from the one described and figured. This mistake was due to the same cause as the preceding, *i. e.*, improperly controlled inoculation experiments.

Yellow saprophytic organisms, in particular, are very common on the surface of plants. Mr. Waite found a yellow non-parasitic Schizomycete associated quite frequently with the pear-blight organism, and this misled him for a time. Dr. Arthur must also have had it in some of his cultures for in one place he describes the growth of *Bacillus amylovorus* as yellowish. Chester has described this white organism as pink.

The writer found yellow non-pathogenic bacteria associated with the yellow *Bacterium malvacearum* on the cotton-plant. In 1908 he obtained a whole series of yellow bacteria from the surface of corn kernels, but not *Bact. stewartii*, which is also yellow, and in many respects like those which were obtained. This corn was undoubtedly infected with *Bact. stewartii*, but in such small numbers as to evade detection by this method.

In crown-gall of peach the writer has frequently found non-parasitic yellow and white bacteria, sometimes to the exclusion of the right organism. Yellow ones were also plated out by O'Gara and inoculated without result. Hedgecock also plated out yellow colonies and inoculated unsuccessfully. The same fact has been observed by the writer in daisy gall, in the rose gall, and in *rogna* of the grape. Once I obtained a nonpathogenic white organism from *rogna* of grape, once also a non-pathogenic white organism from hard gall of the apple, and later a pathogenic one. These yellow bacteria are so common it must be assumed that they have a special aptitude for the decaying tissues of these plants.

In old flabby tumors on the hop sent on from California in February the writer found the deeper tissues swarming with green fluorescent and other non-pathogenic Schizomycetes which must have penetrated from the surface of the plant. The right organism was recovered, but it formed only a very small portion of the total bacterial flora of the tumor. Before cutting into it, the surface of this tumor, which was free from distinct fissures, was scraped, washed, plunged into alcohol and then for six minutes in 1: 1,000 mercuric chloride water so that contamination by organisms lying on the surface was presumed to have been excluded.

The writer has seen potatoes typically rotted by *Bacillus phytophthorus* from which, nevertheless, it was almost impossible to isolate the parasite, its place having been taken by incalculable numbers of nonparasitic, rod-shaped bacteria, which further disintegrated the tissues. In one instance repeated platings from rotting tubers failed, but the right organism was finally obtained indirectly by making a copious inoculation of the diseased flesh into a sound potato, and plating from the latter after decay began. Appel reports a micrococcus as frequently following *Bacillus phytophthorus*, and this is probably what led Dr. Frank astray. A white non-pathogenic coccus sometimes follows *B. tracheiphilus* in cucurbit stems.

Greig Smith has isolated a red micro-organism from sugar-cane attacked by Cobb's disease, and has ascribed the red strands to it. The writer found only yellow bacteria in the red strands.

Haven Mectalf found a non-pathogenic red Schizomycete associated almost constantly with the Piricularia disease of rice in South Carolina. Its habitat is undoubtedly the surface of the rice plant.

In experimenting with peas Mrs. Bitting found that fresh peas taken from the pods under sterile precautions always failed to contaminate culture-media, and that this condition of sterility persisted for a number of hours (fig. 2), but not for days. Peas from pods picked for a longer time than 18 hours, often infected cultures and gave, with lapse of time, an increasingly large number of contaminations, showing that the surface bacteria were able to enter the unopened pods and contaminate the seeds (verbal communication).

In his physiological experiments with germinating seeds of *Vicia faba*, etc., where surface sterility was necessary, Harley H. Bartlett obtained it in many instances by carefully slipping the soaked seeds out of their seed coats.

Düggeli, in Zürich, studied this subject quantitatively as well as qualitatively. He experimented with about 40 species of plants, some of which were sampled repeatedly. He selected sound parts—seeds, fruits, stems, leaves, and whole plants in case of certain seedlings grown in sterile sand. A great many gelatin poured-plates were made, and his detailed studies involved an enormous amount of labor.

In general he found great numbers of bacteria on the surfaces of plants. Only very exceptionally did he fail to obtain them, but occasionally they were few. Shaking the stem or other part in water for ten minutes did not remove all of them. He therefore made his comparative tests by grinding up a measured portion or weighed quantity of the material, some of which then served for the test.

The same organisms were found on seeds, seedlings, and mature plants. In general there was a poverty of species. A few species occurred on so many plants and over and over again so abundantly that he was forced to regard them not as accidental occurrences, i.e., not as organisms which had settled down out of the air, but as true epiphytes peculiar to the surface of the plants.

The most common form was a motile gelatin-softening yellow schizomycete, named by him *Bacterium herbicola aurum*, but said to be the same as the *Bacillus mesentericus aureus* isolated by Winkler from the surface of plum leaves. This form occurred on a great variety of plants, often to the exclusion of other species. For example, of 55 samples of seeds examined, 21 bore this organism, practically to the exclusion of all others, while only 7 samples were free from it. This organism is a short, actively motile, non-sporiferous rod, single or paired, 1 to 3 x 0.6 to 0.7 $\mu$ , forming characteristic zoologae. No statement is made respecting number or attachment of flagella. It is gold-yellow on gelatin and agar (gray at first), and gold-yellow on potato. Milk remained unchanged or was curdled by acid, some acid formed also in the bouillon. Urea was not converted into ammonia. Nitrates were reduced, and there was a strong indol reaction in bouillon 6 days old. The organism is aerobic and probably facultative anaërobic. Growth was good the whole length of the stab, but ordinarily no gas was formed from grape-sugar. It does not stain by Gram. Growth and pigmentation on agar streaks was more rapid at 37° C. than at 30° C.

A second common form was a liquefying, green-fluorescent species, identified by him as *Bacillus fluorescens* (Flügge). A third species, *Bacterium putidum* (Flügge) L. & N. was also found widely distributed. According to Düggeli these three species are the dominating ones on the surfaces of plants.

A fourth common species produces a manganese red pigment on potato. This is called *Bacterium herbicola rubrum*. It was feebly motile and did not liquefy gelatin. Other sorts were much less abundant. Of these he names: *Bacillus mesentericus*, *B. vulgatus*, *B. megaterium*, and *B. coli*.

Some of his experiments showed that the bacteria were carried over from seeds to seedlings. On the surface of the latter they were found in much greater numbers than on the seeds, particularly if the seedlings grew in closed dishes where the air was moist and dew often appeared on their surfaces. The occurrence of the same bacterial forms on the surface of mature leaves and stems suggests the way in which seeds and fruits become contaminated.

The nutritional requirements of his yellow and red species appear to be very simple, since they were able to obtain their carbon food and nitrogen food from many different substances. *B. fluorescens* and *B. putidum* were more exacting in their requirements.

Fig. 4 shows masses of bacteria growing between the petals of the unopened flower of the hothouse carnation in what the writer has called the gum-bud disease of the carnation. They are seen to be entirely outside of the tissues. Subsequently the petals withered and the tissues were penetrated. At first the writer thought he had discovered a genuine bacterial disease, but further study indicated that the growing together of the petals on which depends the inability of the blossom to open normally, is the real cause of the disease and precedes the occurrence of the bacteria. The cause of this fusion of parts normally separate is unknown.

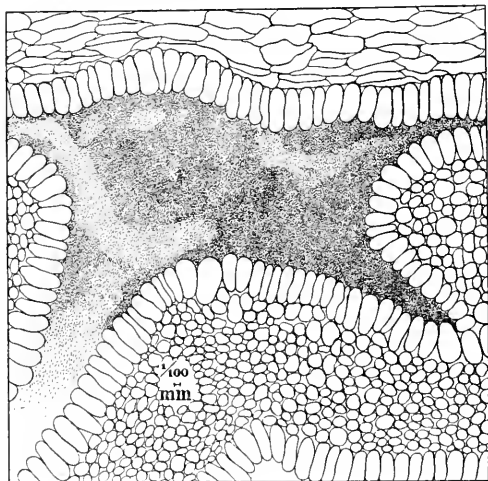


Fig. 4.\*

In 1890, in the *Annales* of the Botanic Garden of Buitenzorg, in a paper on the flower buds of *Spathodea campanulata*, Dr. Treub mentions the fact that various bacteria occur normally in the liquid secreted inside of the closed calyx. His statement is as follows:

The relatively large quantity of organic product [in the excreted fluid] explains the very curious fact, that normally colonies of different micro-organisms develop in the liquid of the pitchers of the *Spathodea* without appearing to injure in any manner by their presence the floral organs in the process of development. The introduction of the microbes producing these colonies may take place in two different ways: first, they may date from the time when the very young calyx is still open; then, they may insinuate themselves later into the narrow canal at the summit of the pitcher and thus in the end arrive at the liquid. I am inclined to believe the second mode of introduction

is the more frequent. In view of this fact it is not astonishing that the liquid contents of the perianth of the *Spathodea campanulata* is more or less putrid and ammoniacal.

In 1897, in a long paper on the *Hydatodes* of the blossom buds of some tropical plants, Mr. S. H. Korrders, in the same *Annales*, also refers to the "constant occurrence of bacteria or fungus threads in the interior of water calyces." He mentions bacteria as occurring in the flower buds of *Spathodea campanulata*, *Clerodendron minahassae*, and *Kigelia pinnata*. In the flower buds of other plants he found fungi. So far as he was able to observe, only one fungus species occurred in one sort of water-calyx plant, although one would naturally expect mixtures. In case of bacterial growths the reaction of the fluid inside the calyx

\*Fig. 4.—Gum-bud disease of carnations. Cross-section of a Scott carnation, showing bacteria lying between outer petals of unopened bud. The petals are stuck together (grown together in many cases) and unable to open. Bacteria not in the tissues, and cause of disease unknown. Syringe-water contaminated by manure-water. March 1903. Glen Burnie, Md.

was alkaline. In case of fungus growths the reaction was acid. The constant occurrence of the bacteria or of the fungi in the interior of the water calyx in no way injures the host plant. The author ascribes the presence of bacteria or fungi to alkaline or acid secretions of the plant, but inasmuch as he seems to have determined the reaction only after the organisms had grown in the fluid, his conclusion does not necessarily follow.

These organisms are really still outside of the plant. Their presence here, while extremely interesting, is only to be compared with what occurs in the mouth-secretions of animals, and it is doubtful if there is any true symbiosis, *i. e.*, mutual profit.

The most recent reference to this subject, so far as known to the writer, occurs in an address by M. C. Potter (Trans. British Myc. Soc., 1910, Vol. III, p. 110) from which fig. 5 is borrowed. He found numerous bacteria on the surface of leaves (potato, artichoke, etc.), and cultivated them therefrom by making impressions of the leaves on gelatin plates.



Fig. 5.\*

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\*FIG. 5.—Impression of leaf of *Helianthus tuberosus* on gelatin after 3 days' incubation. The form of the leaf is outlined by the development of numerous bacterial colonies, germs of which were present on the surface of the leaf.

THE ENTRANCE OF BACTERIA INTO PLANTS—THE QUESTION OF PARASITISM—  
WHAT CONSTITUTES A PARASITE.

Among various writers there appears to be more or less confusion of ideas on the subject of bacterial parasitism in plants, and on the relative importance of the various factors concerned in the production of disease. This has arisen partly from a confusion of terms and partly from ignorance of the facts. Wehmer's argument that "bacterial decay is only the last stage of the injury begun by environment," proves too much. It applies equally well to animal diseases and, if pushed to its logical extremity, ends in a *reductio ad absurdum*. No one denies that the host-plant or host-animal is more or less favorably disposed for the attack of a bacterium according to a variety of external circumstances influencing growth, nutrition, and vigor. Some disturbance of the normally acid condition of the stomach allows the cholera vibrio or typhoid bacillus to pass undestroyed into the alkaline intestine where it thrives. A slight rawness of the throat, with the death of a few cells under certain bodily conditions, allows the diphtheria or tubercle organism to obtain a foothold. A wound so insignificant as to be given scarcely a second thought, affords an opportunity for arthritis, septicæmia, anthrax, plague or tetanus to develop. Are these and similar diseases any the less diseases due to bacteria because the entrance of the pathogenic organism was favored by some wound or exudate, or other abnormal condition of the host? By no means! Under ordinary conditions the dead cells would be sloughed off, the wounds would heal, and the organism as a whole would continue in its normal physiological course. The introduction of the pathogenic organism is the real fundament of the situation. It begins a new train of phenomena, and by no amount of argument can it be made clear to practical men that, to quote again from Wehmer, "the first sort of decay," *i. e.*, the primary lesion which allowed the bacterium to gain an entrance, "is of interest practically to the exclusion of the other." To the individual who experiences it, the puncture of a pin or thrust of a knife which ends in tetanus must always be of considerably more importance than one which is followed only by temporary pain or inconvenience, and so of every other injury ending in a bacterial disease.

It is not denied that general conditions of the host-plant or animal are at times especially favorable to the development of the disease, and at other times unfavorable. Neither is it denied that the death of a few cells, by suffocation or otherwise, may afford just the necessary foothold for the beginning of a destructive disease. These may be the predisposing causes, but they are not the actual cause, neither has the fact that there are predisposing causes been generally overlooked by animal or plant pathologists, although it must be admitted that they are often difficult to disentangle from a multitude of non-essentials and to bring into clear relief. There are many degrees of individual predisposition to parasitism, as De Bary pointed out long ago, and as every working pathologist recognizes.† The exact determination of the factors leading to special predisposition

†As may be seen from the following paragraph, Reinke and Berthold pointed out as long ago as 1879 that some potato tubers are much more subject to bacterial rot than others:

Differenzen im Widerstands-Vermögen gegen die Infection zeigen sich aber auch zwischen verschiedenen Knollen, welche vollkommen frei von Phytophthora sind. Wir haben beobachtet, dass solche Knollen, die in einer Wundgeimpft und unter eine Glasglocke gelegt waren, binnen zwei Tagen sich vollständig in eine jauchige Masse umgewandelt hatten, während andere die Bacterien nur auf kurze Strecke in das Parenchym eindringen liessen, und dann die nassfaule Stelle durch eine Korkplatte aus ihrem Organismus auszuschneiden wussten. Jedenfalls müssen zwei Momente zusammentreffen, ein äusseres und ein inneres, damit die Nassfäule in einer Kartoffel ihren rapiden Verlauf gewinne, welcher bereits in wenig Tagen zur vollständigen Auflösung führt; erstens inficirende Bacterien mit ihren Fermenten, und zweitens Disposition des Kartoffel-Individuums. Wo sich in letzterer Hinsicht verschiedene, ausgereiften Kartoffeln Unterschiede zeigen, da sind dieselben theilweise vielleicht in der Race begründet; es wäre denkbar, dass die wasserreicheren, stärkeärmeren Sorten leichter der Zersetzung zur Beute fallen. Doch muss diese Frage durch speziell darauf gerichtete, ausgedehnte Versuchsreihen entschieden werden.

to disease is a work for the future, and our most brilliant pathological successes will lie in this direction. It is, however, a task for generations.

H. Marshall Ward's view, as expressed in his book on *Disease in Plants*, is also untenable. This is that the parasitism of bacteria in plants must be held in doubt until it has been proved that they enter living cells in the same way as certain fungi, *i.e.*, by enzymic action, as De Bary proved the penetration of cell-walls by certain fungi.\*

The question is purely a verbal one, *i.e.*, one of definitions. If a bacterium, or any other organism for that matter, is to be considered a parasite only in so far as it conforms to Marshall Ward's definition, then the case is adjudicated. Admitting the premises the conclusion follows, and no more remains to be said. The error lies in the narrowness of the original definition, which admits for a parasite only one mode of action, and which would exclude tetanus or anthrax as effectually as pear-blight or olive-tubercle from any list of parasitic diseases.

What is a parasite? The word is from two Greek words *para*, beside, and *sitos*, food, and is very ancient. It means literally, we are told, *one who eats with another*. It came, however, in course of time to have a bad meaning. It was used by the Greeks to designate certain hangers-on at feasts who came uninvited, devoured the food and gave nothing in return but fawning and flattery. With lapse of time the word has acquired various secondary meanings. There has been considerable change of view even since 1884, when De Bary discussed the subject in his classical *Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien*. To-day, in a broad way, a parasite may be defined as an organism which is nourished at the expense of another organism and which gives little or nothing in return. The last part of the definition appears to be required to exclude the relations of mother and child and various real or supposed cases of symbiosis, *e.g.*, lichens, root-nodules of Leguminosae, etc. The following somewhat narrower definition excludes the old Greek idea and its modern equivalents, but is equally applicable to the subject in hand: A parasite is any organism which passes the whole or a part of its life cycle on or within another unlike organism deriving its food therefrom and injuring it in the process. A parasite might also be defined as an organism living with and taking its nourishment from another organism, and by this union causing a disease, weakening, or malformation in the attacked plant or animal.

In the past there was sometimes added to the definition a statement that the parasite was unable to obtain its food from nonliving substrata. Experiment, however, has demonstrated that some of the supposedly strict parasites, those which De Bary designates as *obligate parasites*, are able to grow on nonliving media. In other words, one after another of these organisms has been transferred to the group of facultative saprophytes, while many supposedly pure saprophytes have been found to be facultative parasites, *e.g.*, many species of *Fusarium* and the *Bacillus coli*. It is probable, therefore, that if this portion of the definition is insisted upon *de rigueur* we shall in the end be reduced to the ridiculous situation of having no parasites at all, since at no distant time it is likely that ways will be found of cultivating all of the so-called strict parasites on artificial media. There would then certainly have to be some shifting of out-worn definitions, but the facts in the case would remain the same, the effects of certain bacteria on living plant and animal tissues would not then be more destructive than they are now.

The direct injury caused by a parasite may be extremely slight, *i.e.*, confined to a few cells, or may be so extensive as to involve many systems of tissues. The indirect injury in plants usually bears a rather close relation to the direct injury, *i.e.*, to the extent of multiplication of the organism, but in some of the animal diseases by reason of toxins it is out of all proportion to the actual multiplication of the parasite, *e.g.*, in diphtheria and

\*The actual statement is as follows: "but it is necessary to bear in mind that actual penetration of the cell-walls from without must be proved as De Bary proved it for the germ-tubes of fungi, before the evidence that bacteria are truly parasitic in living plants can be called decisive."

tetanus. Nothing, for instance, is yet known among bacterial diseases of plants comparable to the action of the tetanus poison. When large limbs of trees are destroyed, without the general distribution of the bacteria in these limbs, as in pear-blight, death results from the girdling action of the organism lower down upon the limb or trunk and is due to a mechanical injury exactly as if the limb were ligated or peeled. The whole field, however, has not been worked over.

There are many grades of plant parasites from those which appear to require only the slightest foothold, even in vigorous subjects, to those able to attack only under conditions of depression or during that weakness of age preceding natural decay. In this particular, plant-diseases do not differ materially from animal-diseases. Probably malnutrition plays a large part in rendering plants and animals susceptible to disease, but when we come down to specific details and proper dietaries we are still very much in the dark, largely, it may be presumed, from the slowly cumulative effect of such influences and the lack of sufficient experimentation. Very vigorous looking plants and animals often succumb to disease. Yet even here appearances may be deceptive, and it is safe to say that in a few decades we shall know much more than we do at present about what really constitutes *vigor* in the sense of resistance to disease. We are now probably often deceived by appearances, designating as *vigorous*, both plants and animals which, under adverse circumstances, would really have very little power of resistance. We know already that rapidly growing, luxuriantly green plants have frequently had too much nitrogen and are in a worse condition, *i. e.*, less able to resist cold and certain diseases, than paler green, slower growing individuals. It is also believed by some that the presence in the soil of an abundance of lime and phosphates renders certain plants hardy. In case of plots of potatoes grown on the Potomac Flats in Washington, and treated heavily for two years at planting time with various standard fertilizers, *c. g.*, lime, potash salts, phosphates, nitrates, etc., and subsequently inoculated in the foliage and green shoots with various bacteria, *Bact. solanacearum*, *Bacillus coli*, the writer could not see that the previous treatment of the soil made any difference in the sensitiveness of the plants grown upon it. The subject, however, is one which invites experiment.\*

To be a parasite then, it is not necessary that injury to the host should come about in one specific way. As a thief may enter a house through the cellar or the roof and by way of an open door or a closed window, the essential thing being the fact of entrance and theft, so two parasitic organisms may attain the same end by two quite different ways. The organism may gain an entrance in any way it can, and may abstract its food from the host-plant in any way most congenial to it, either by ramifying exclusively in the intercellular spaces and middle lamellæ, by growing through the cells, by sending haustoria into the cells or by secreting enzymes or toxins which destroy the cells, the substances of which are then used for its growth, whereupon fresh enzymes or toxins are secreted for the destruction of remoter cells to be in turn converted to the uses of the ever multiplying hostile organism. Such, at least, is my conception of a parasite and such is my use of the word. Those who wish may hold on to the old terminology, or any terminology they desire. That any bacteria causing diseases in plants are "streng obligate Parasiten," to use De Bary's term, I have never maintained, neither do I believe that there are any such parasites whatsoever. We may retain the term, if we like, but probably it is only a convenient expression to cover our ignorance.

That bacteria can enter the host-plant in the absence of visible wounds is no longer a matter of doubt. They do not enter by the enzymic action of hyphal filaments, or germ-tubes, because they do not possess such organs, but they do so in an equivalent way; that is, they attack the plant through natural openings, destroying the nearest cells first, and

\*Recently Lyman J. Briggs, of the U. S. Department of Agriculture has shown that by withholding lime and potash and adding acid phosphate at the rate of 1,000 lbs. per acre a serious disease of tobacco in Connecticut (due to the soil fungus, *Thielavia basicola*) can be prevented almost entirely.



then, as they continue to multiply, those that are more remote. They do this in the same way as certain fungi, *i. e.*, by the disintegrating action of secreted soluble substances—enzymes, etc.

The question might still be raised legitimately whether they can ever enter the plant, or the animal for that matter, except when favored by some slight mechanical injury, *e. g.*, the death of a few cells by asphyxiation, or otherwise, to offer a starting pabulum. So far as we know, they enter water-pores and stomata, with subsequent infection of the plant, only in the presence of water, but in this respect they are not different from the fungi. All we know definitely is that they enter the plant under conditions that are very common in nature and that they set up grave disturbances, whereas, if they are not present, the drops of water disappear from the surface of leaves and stems and the plants remain unharmed. In nectarial, stomatal, and water-pore infections, which are extremely common in certain diseases, wounds in the ordinary meaning of that word are out of question. It is conceivable that rain-drops or dew-drops might remain on the plant long enough to kill or injure certain cells, which would then extrude fluids and furnish the slight amount of food required by the bacterium to begin operations. This is not impossible and may even be considered as perhaps probable in certain diseases of rainy seasons, *e. g.*, pelargonium leaf spot; but it has not been established for any disease, and that it is a necessary postulate in all cases seems unlikely for several reasons. In some of my spraying experiments with *Bacterium maltaccarum* on cotton, small round spots ascribed to suffocation appeared on some of the leaves, but these were just the places which did not contract the angular leaf-spot. The latter appeared later in other places on the leaves as a result of stomatal infections. The writer has proven conclusively in at least one case, *viz.*, the black rot of cabbage, that the bulk of the infections take place through the water-pores, and that the fluid extruded naturally from these substomatic chambers contains food enough to enable *Bact. campestre* to begin its growth. This is all that is required. In case also of *Bacillus amylovorus*, causing the fire blight of pome fruits, the nectar of pear and apple flowers affords all the food necessary for the beginnings of growth and of destructive action on the neighboring cells.

Wehmer asserts that the tissues of the potato-tuber are always asphyxiated before bacterial invasion occurs, but he appears to have experimented only with saprophytes, and has not established his contention. In Appel's experiments and also in my own the soundest potato tubers in the driest air available in the laboratory have been rotted rapidly by inoculating them with *Bacillus phytophthorus*. In the brown rot of potatoes due to *Bact. solanacearum* it is not necessary that the tubers should be wetted or wounded to become infected since this bacterium is capable of passing from the stems into the tubers by way of the vascular bundles of the rhizome, coming to the surface only in late stages of the disease.

The objections to bacterial parasitism in plants have been objections coming from those not familiar with such phenomena, and we all know how difficult it is at first for new ideas to make their way. Such things could not happen because they had not come within the ken of the objector, or because the physical nature of plant-tissues offered (theoretically) an insuperable obstacle to their multiplication, or because plant juices were acid and all known bacteria required an alkaline medium, or because if such diseases existed, one would already have discovered them. All of these objections were the result of inductions based on insufficient evidence. A thousand observations, let us say, confirmed them, but then the thousand and *first* upset them completely.

It was found that some bacteria could live in acid media, and that others could convert acid substrata into alkaline by methods of their own. The chemical objection therefore was removed. The physical one proved to be founded upon a misconception of the mode of action of these organisms. There remained, therefore, as the sole basis for scepticism the inertia of accumulated disbelief, the ingrained views of a generation, and, finally, the

important fact that an unusually large number of poor researches in this field obscured the general view and covered the whole subject so to speak, with a wet blanket.

#### THE CARRIERS OF INFECTION.

This subject is now widely investigated and popular, particularly in relation to the spread of human diseases, but when the first evidence was obtained showing that bacterial diseases of plants are transmitted by insects almost nothing was known.

The first exact experiments were by Merton B. Waite in 1891. That year he proved conclusively that pear-blight is disseminated by bees in course of their visits to pear blossoms for nectar and pollen.

In 1893, the writer obtained some evidence that the bacterial wilt of cucurbits is transmitted by beetles, and some years later established the fact conclusively.

In 1895, the writer obtained very typical cases of the bacterial brown rot of the potato using the Colorado potato beetle as the agent of transmission.

In 1897, the writer showed that the bacterial black rot of crucifers could be transmitted by insect larvae (*Plusia*) and by molluscs (*Agriolimax*) and pointed out that there was no evidence of transmission of the disease by wind. Brenner confirmed a part of this and incriminated aphides.

More recently gall-forming nematodes were observed by Hunger in Java (in 1901), and by the writer in the United States (in 1908), to function as carriers of a bacterial disease of tomato, tobacco, etc.

In 1910, D. H. Jones, in Canada, proved apple-blight (*Bacillus amylovorus*), to be disseminated from diseased to healthy shoots by aphides and by bark-boring beetles (*Scolytus*).

There is therefore every reason to believe that small animals play a large part in the dissemination of these destructive diseases. Elsewhere full details are given.

#### SPECIFIC DISEASES.

Admitting the parasitism of bacteria in plants, are there any specific diseases? The physician depends to a considerable extent on subjective symptoms for his diagnoses. Headaches, pain in various organs, etc., give him many clues. He also has his clinical thermometer. There is nothing in plants, however, so far as we know, corresponding to the rise in temperature which we call fever. The plant pathologist must depend entirely on objective signs—spots, stripes, distortions, enlargements, atrophy, yellowing, sudden wilting, etc. Moreover, the plant body being much less highly organized than the body of man and the domestic animals, one might expect less differentiation in objective signs due to the action of various parasites and to a certain extent this is true. For instance the soft-rot bacteria all produce much the same set of phenomena and are capable of attacking plants belonging to widely separate groups. There are other organisms, however, that seem to be restricted to particular families and the morbid phenomena which they originate can scarcely be mistaken for diseases due to any other micro-organism. Pear-blight is a good example of such a disease. We know only one organism capable of causing this train of phenomena. In like manner, so far as we know, only one organism is able to cause the bacterial wilt of cucumber, only one is able to cause the olive-tubercle. These three diseases are restricted so far as known, to as many families of plants, and there are also restrictions within each family, not all genera or species being susceptible. In this respect the causes of these three diseases are very different from the soft-rot organisms, the action of many species of which overlap, *e. g.*, we may have a soft-rot of the potato or cucumber due to half a dozen different organisms, the signs being essentially the same. From this point of view these, therefore, are the lowest type of bacterial parasites. A third type of organism is able to produce quite specific over-growth phenomena in a

great variety of plants. The best example we have is the recently discovered crown-gall bacterium. So far as we yet know, *Bact. tumefaciens* is the only organism capable of producing the crown-gall, but it can do this in plants belonging to an astonishingly large number of families, *i. e.*, in not less than 18 widely separated ones. In other words, it is an organism, or a closely related group of organisms, with a very generalized and simple set of requirements such as many plants are able to offer. Diseases superficially resembling crown-gall may, however, be produced by a number of organisms, *e. g.*, on sugar beet by *Bacterium beticola*, on olive by *Bacterium savastanoi*.

In the matter of the experimental production of parasites, or better, let us say, in the testing of all sorts of bacteria in all sorts of plants to learn their behavior, we are only at the beginning, and some statements already made must be taken with a grain of salt.

Some useful knowledge would undoubtedly result from systematic experiments of this sort, but much time and labor would be necessary to go over the field even in a cursory way. During such inquiries it is not unlikely that one might stumble upon certain active parasites, cell-wall destroyers, etc., but the waste of time in testing a miscellaneous lot of organisms would be very considerable, and after all it might be questioned very properly whether this is really the best way to approach the problem. A better way would be to begin with organisms known or suspected to have particular actions, and first determine the nature and extent of these actions. With this end in view, the writer has been in the habit of taking organisms known to be pathogenic to certain plants and testing them in a variety of other plants to determine their behavior. He has also made some experiments with saprophytic forms. Nothing has been observed, however, which favors the view that non-parasitic forms can be induced readily to adopt a parasitic life. To be or to become a parasite an organism must be endowed with certain peculiarities adapting it to conditions as they occur in particular plants or animals. The environment must be congenial. An organism may never have functioned as a parasite, but if it possess these necessary peculiarities it is capable of becoming one when introduced into the plant or animal and then we shall have a new disease. Such an organism is from the beginning a parasite *in posse*, if not *in esse*, and it is only our ignorance of such facts that would ever lead us to suppose that we can easily convert all sorts of saprophytes into parasites.

The most important papers are those of Laurent, Lepoutre, van Hall, and Jensen. (See also individual and varietal resistance.) The subject is so vital that I have abstracted papers by the above named writers at some length.

#### THE EXPERIMENTAL PRODUCTION OF PARASITES.

In 1899, Laurent published an account of his experimental researches upon diseases of plants, dealing especially with the influence of foods upon the resistance of plants to parasites.

His field of experiment was in good clayey soil, containing, according to an analysis:

Organic matter	54.40 per cent.
Total nitrogen	1.70 per cent.
Lime	15.60 per cent.
Potash	0.96 per cent.
Phosphoric acid	3.03 per cent.

Four equal plots were laid out, and received the following doses of fertilizer per hectare:

- Plot I. 1100 kg. of sulphate of ammonia.
- Plot II. 2200 kg. of kainite, containing 13 per cent of anhydrous potash.
- Plot III. 2200 kg. of superphosphate of lime, containing 15 per cent of anhydrous phosphoric acid.
- Plot IV. 15,500 kg. of quick lime (chaux grasse).

Upon each plot potatoes (var. Simson) and carrots (var. Nantaise), and other species, were planted early in April. The tubers and roots harvested in October were used for experiment the following February.

Slices of both potatoes and carrots from the four plots were placed under a moist bell jar and were sowed with conidia of *Bolrytis cinerea* which had been cultivated upon gelatinized must of

beer. The mould did not develop, but on a slice of carrot from plot IV, there appeared a little bacterial colony of a ropy consistency, made up of short bacilli, introduced probably when the conidia were sowed. All his attention was then focussed on this bacillus.

Inoculations were made with a flamed scalpel on slices of carrot kept at laboratory temperature. Sections from plots II and IV became infected in 4 days while those from I and III remained uninjured. Two later attempts to inoculate I and III failed.

Inoculations of slices of carrots from all plots, using the microbe obtained from IV of the preceding series, gave positive results. Another series using material from plot II of the second series gave a general development on roots from plots I, II and IV, but scarcely any colonies on those from III.

A fourth series, however, inoculated with bacteria taken from slices of plot I (third series, kept at a temperature of 25° C.) gave a growth on all the slices from all the plots.

Thus the microbe had become parasitic even upon the most resistant carrots after three passages through less resistant ones.

Results absolutely comparable were obtained with tubers of potatoes: Thus, tubers from plot IV were readily attacked, those from plots I and III less so, while those from plot II were successfully inoculated only after four passages of the bacillus through tubers from plot IV.

When the microbe grew it finally transformed the invaded tissues into a pulp, composed of disassociated cells in which the starch-grains persisted. The cylindrical bacilli swarmed around and finally within the cells.

This schizomycete was identified as *B. fluorescens putidus*. It was readily cultivated in a mineral solution:

Water.....	1,000.
Neutral ammonium phosphate.....	2.5
Neutral potassium phosphate.....	2.5
Magnesium sulphate.....	1.0

to which had been added various organic matters—sugars; alcohols; peptone; asparagin; succinate, lactate, citrate and tartrate of potassium, etc.—but in such cultures it lost its virulence, to such an extent that inoculations on tubers from plot IV gave negative results. Only by diminishing the resistance of the potato cells by the use of alkaline solutions was infection made possible.

In March, a new series of experiments was begun, the four plots receiving fertilizers per hectare as follows:

- Plot I. 500 kg. sodium nitrate and 800 kg. sulphate of ammonia.
- Plot II. 2,000 kg. kainite, containing 13 per cent of potash.
- Plot III. 2,000 kg. superphosphate of lime, containing 15 per cent of phosphoric acid.
- Plot IV. 40,000 kg. quick lime.

A fifth plot received 2,750 kg. sodium chloride per hectare. This was to determine what effects might be attributed to the osmotic action of large quantities of soluble salts. In a sixth plot the plants mentioned below were cultivated without special fertilizers: Eight varieties of potatoes were used, namely three which had the reputation of being subject to disease, viz., Marjolin, Early Rose, and Blanchard; three considered to be resistant, viz., Clave, Simson and Chardon; and two other sorts, viz., Pousse Debout and Zeland. The tubers of Simson used as seed were harvested from the corresponding plots of the previous year. In addition to potatoes, Nantes carrot, Witloof chicory, Jerusalem artichoke and a local variety of sugar beet were cultivated on these plots. All the seed-tubers sprouted and developed regularly, but in plot V the salt plainly injured the germination of seeds.

At the time for inoculation, the fluorescent bacillus was found to be lacking in virulence as it had been kept on artificial media. Therefore, Laurent undertook to get the bacillus as in the first place, but obtained this time a motile nonfluorescent organism (2 to 5 x 0.5 to 0.6  $\mu$ ) the colonies of which it is said resembled those of *Bacillus coli*. Submerged colonies were little yellowish disks, while surface ones were pearly white, spreading with a circular or sinuous border.

The different varieties of potatoes yielded very differently in the several plots, showing that each variety has its own requirements as regards mineral foods.

Halves of tubers of Marjolin from all the plots were inoculated with the bacillus from tubers of plot IV, and kept in the thermostat at 30°. All were attacked within 6 hours, but while penetration continued in sections from plot IV, reaching 10 to 12 mm. by the fourth day, it ceased in all the others by the second day, with a healing of the spots.

Slices of Early Rose from all plots, inoculated with cultures from tubers of plot IV in the preceding experiment and kept at 30°, offered little resistance to the disease, except in tubers from plot V where the sticky surface layer reached a thickness of 4 to 5 mm.

From other experiments the following general results were obtained:

The variety Blanchard is very sensitive. Pousse Debout is very resistant. Tubers of Chave from I and IV were most attacked, those from V least. Tubers of Chardon from IV were seriously injured, those from I and II only a little, and those from III not at all. In tubers from plots I and IV a black zone was observed between the attacked and the healthy tissues. As this stain was not noticed elsewhere, Laurent attributed it to the nitrogenous product formed by the bacteria at the expense of the tissues.

Corresponding experiments made at 20° to 22° C. on Simson and Chardon potatoes and chicory and carrot, gave very similar results. The inoculating material came from a third passage through Early Rose, and was, therefore, quite virulent. All tubers and roots from plot IV were rapidly attacked. The variety Simson was much less resistant to this bacillus than to *B. fluorescens putidus*. Carrots from plots V and III and chicory from plots I, V, and III, resisted most strongly, while those from IV were always completely attacked.

Comparative experiments were also made on tubers coming from (1) a field dosed with 800 kg. sulphate of ammonia, 800 kg., superphosphate, and 400 kg. each of kainite and sulphate of lime and (2) a field which had received 80,000 kg. of barnyard manure. Tubers from (1) rotted completely at 35° within 5 days after inoculation with virulent bacilli. Those from (2) rotted, but less rapidly. Tubers from unfertilized land resisted the rot better than either of the other lots. Hence, Laurent concludes that the use of fertilizers, by allowing an exaggerated absorption of nitrogenous compounds, favored bacterial invasion. He states that lime diminishes the resistance of the potato, carrot, and chicory. Nitrogenous fertilizers and potash salts had analogous but less striking effects. On the other hand, phosphates, and to a lesser degree sodium chloride, increased the resistance.

The results with lime led Laurent to believe that differences in resistance were due to a modification in the acidity of the cell-sap. Experiments were undertaken to test this as follows:

Tubers of Chave and Chardon, from plot III, known to be resistant, were cut in two and plunged for 5 hours in the following solutions made up with distilled water:

Potassium sulphate.....	2 per cent.
Calcium sulphate.....	1 per cent.
Ammonium sulphate.....	2 per cent.
Asparagin.....	2 per cent.

At the same time tubers of Early Rose and Marjolin from plot IV, known to be sensitive to rot, were similarly plunged in the following solutions:

Neutral sodium phosphate.....	2 per cent.
Neutral potassium phosphate.....	2 per cent.
Neutral ammonium phosphate.....	2 per cent.

All the tubers were inoculated with the bacillus from a fourth passage through Early Rose, and were kept in the thermostat at 35°. The first lot (resistant varieties) was uninjured, the second lot (sensitive variety) was attacked. Exposure to these solutions, therefore, did not alter the resistance of these varieties. Even exposure of the cut flesh of Marjolin and Blanchard for 40 hours to 1 per cent acid potassium phosphate did not protect them when inoculated; after 15 hours at 30° C. they were badly rotted.

Resistant tubers of Chave and Chardon from plot III were immersed 3 hours in lime water, 1 per cent potash or 1 per cent soda solutions. At the same time sensitive tubers of Early Rose and Marjolin from plot I were plunged in 1 per cent solutions of tartaric, citric, and lactic acid. All were then inoculated with the bacillus from a fifth passage through Early Rose. After 12 hours at 35° all were infected; the alkalis rendered the resistant ones susceptible and the acids did not protect the sensitive ones. When the dose of organic acids was increased, however, the organism did not succeed in penetrating the tissues, the acidity of whose cell-sap was thus artificially increased. This is supposed to be due to the fact that the enzyme which dissolves the middle lamellæ, acts on the potato only in a slightly acid or else in an alkaline medium.

As shown above, the cut surfaces of resistant tubers were rendered sensitive by immersion in 1 per cent alkaline solutions. The total acidity of the cell-sap does not, however, furnish an indication regarding the mechanism of immunity, for tubers of Preciosa and Zeland, two refractory varieties, have an acidity (tested by phenolphthalein and estimated in milligrams of sulphuric acid per 100 cc.) expressed by 231.1 and 289.6, while Blanchard and Early Rose, two little resistant varieties, have an acidity represented by 317.8 and 387.1.

Experiments were then made by immersing slices of little resistant varieties of potato for 12 hours in the juice of two resistant ones obtained by great pressure (300 atmospheres) to test the protective effect of this juice. The results obtained were somewhat contradictory. The juices

tested were boiled and unboiled. They were exposed to the oxydizing action of the air. The slices of potato after exposure for 12 hours in these juices were inoculated with pulp from a rotting tuber. Blanchard and Early Rose rotted readily. Simson exposed to the juice of Zeland rotted some, Simson exposed to the juice of Preciosa (cooked and uncooked) did not rot. Zeland exposed to the juice of Preciosa (cooked and uncooked) did not rot. Zeland exposed to its own juice (raw) lost its natural immunity and rotted, but resisted after exposure to its own juice *cooked*. The most interesting result is the supposed immunity acquired by Simson on soaking in the juice of Preciosa. (This conclusion seems to have been based upon a single experiment.)

His general conclusion is that the resistance of potato tubers is due to the existence of some soluble substances in the cell-sap, the rôle of which can be destroyed by alkaline solutions. The total acidity of the juice of the tubers does not correspond to the action of these protecting substances.\*

The bacillus in question, *i. e.*, that identified as *B. coli*, is very widely distributed, rarely capable of living parasitically on tubers of potatoes, and then only when the tubers have been deprived of resistance by exceptional cultural conditions. Its virulence varies greatly under different conditions. In no case were infections secured on normal tubers or roots when the inoculation material was taken from artificial cultures, even a passage through a slice of cooked potato suffices to suppress the parasitic tendency. The virulence does not continue to increase after 5 or 6 passages through raw potato. Laurent gives a list of 30 compounds from which this organism was able to take its carbon food, and a list of 14 from which it could not obtain carbon.

"Tous les mélanges organiques que je viens d'énumérer, et dans lesquels le bacille s'est développé, ont été déposés en quantité notable à la surface de tubercules de Marjolin coupés en deux, et légèrement excavés afin d'empêcher le liquide ensemencé de tomber. Pour beaucoup de solutions, les essais ont été répétés plusieurs fois en variant les concentrations. Jamais le bacille ainsi cultivé ne s'est développé sur tubercules vivants qui n'avaient subi aucune préparation spéciale.

"Mêmes résultats lorsqu'aux tubercules de Marjolin, on a substitué ceux de variétés Early Rose et Blanchard, cependant si peu résistants."

As already stated, the result was quite otherwise when portions of these cultures were placed on the cut surface of tubers previously treated with 1 per cent caustic soda or potassa.

Light lessens the virulence: Thus tubers of Marjolin inoculated with bacilli from the thirteenth passage, placed under a bell-jar in the sunlight, remained intact even after the cultures were replaced in the thermostat.

Heat beyond a certain point diminishes and even suppresses the virulence. Heating for 10 minutes at 45° and 50° does not retard development, but when 55° and 60° of heat were used, the small colonies which started growth soon ceased to grow and the tuber healed. The bacillus, however, can not attack the potato at a temperature of 40° C., although it grows up to 45°.

Passage through different kinds of roots decreases the virulence. Thus after passing through the acid media afforded by turnips, radishes, or onions, the bacillus seems unable to secrete the alkaline substance necessary for the destruction of the middle lamellæ of potato.

Inoculations on various plants, roots, stems, and fleshy leaves gave slight development only around the point of inoculation. In the inoculated *Opuntia*, however, large brown, decayed spots appeared and the whole plant was finally destroyed.

A section through a diseased tuber showed between the pulp and the healthy tissues a zone free from bacteria yet beginning to disorganize. This was due probably to secretions from the bacteria.

\*Averna-Sacca, who studied in Italy (St. Sp. Ag., 1910) the resistance of grape leaves to *Oidium*, *Peronospora*, and *Erisine* found the more resistant sorts had the greatest acidity of cell-sap. The acidity of the leaves expressed in terms of tartaric acid was as follows:

Sorts.	Per cent of dry weight.
Average of 19 resistant varieties (Rupestris, Riparia, Berlandieri) . . . . .	6.565
Average of 31 non-resistant varieties (mostly European sorts) . . . . .	1.372

An examination of the acidity of the must gave equally striking results:

Grapes tested.	Per cent of acid in the must.
Average of 7 resistant American varieties . . . . .	20.811
Average of 10 non-resistant European varieties . . . . .	7.895

On a field rich in lime these diseases were more prevalent than on a sandy loamy field and the quantity of acid in vine leaves from the sandy field was double that in leaves from the other field.

The author's conclusions are: (1) The resistance of the grape vine to the attack of parasites must be ascribed to the acidity of the juice of their organs: (2) This resistance is not stable but may undergo changes with cultivation or even be annulled completely, wild varieties being most resistant.

The pulp of an infected potato was mixed with water and filtered through the Chamberland bougie. The reaction was distinctly alkaline. After neutralization with HCl, it was divided into 12 parts, 2 of which were used as checks. To the others was added 0.5 per cent of one of the following acids: formic, acetic, tartaric, or lactic, or an equal quantity of soda, and a drop of essence of mustard to prevent the growth of microbes. Pieces of potato were immersed in these. After 12 hours the cells of the checks were disassociated to a depth of 2 to 3 mm. and their protoplasm contracted. The use of 0.5 per cent tartaric acid and 0.5 per cent and 1 per cent soda gave the same result. In the case of 1 per cent tartaric acid, 0.5 per cent lactic acid, 0.5 per cent and 1 per cent acetic acid, the disintegration was feeble, and in other cases the tissues were not attacked. Turnips were affected more rapidly and completely. The reaction given by the pulp and the liquid when diluted in water varies with the nature of the plant attacked—radish and turnip pulp gave an acid reaction, and rotted onion was still more acid.

Active soluble substances are secreted by the microbe in cultures in organic solutions as well as in the tubers.

Exposure to a temperature of 62° for 5 minutes or to sunlight for 8 hours destroyed the solvent activity of the diluted pulp of infected potatoes.

The existence of a variety of cytase was established. Flocculent alcoholic precipitates, dissolved in distilled water, and in the presence of essence of mustard caused the characteristic softening of the tissues of potatoes which were kept in it 12 hours. It acts on the potato in an alkaline but not in an acid medium.

The substances causing the death of the protoplasm were not determined by Laurent. The alkaline secretions which kill the protoplasm are more resistant to heat than the cytase, but are destroyed at 100°. Even after the destruction of these substances the juice is able to diminish the resistance of the most rebellious varieties of potatoes. This indicates that toxic substances are still present.

Laurent states that the second bacillus used in his experiments is a form of *Bacillus coli*. It does not liquefy gelatin, and emits gas bubbles when inoculated by pricking into must of beer gelatin. It develops better in the presence of oxygen than in vacuo, but is anaërobic to a certain extent. Deprived of air, it reduces the nitrates with great rapidity. Several races of this organism were isolated by the author. The essential characteristics of the bacillus of all these races are not modified in any lasting manner by cultivation in bouillon, on cooked slices of potato, on must of beer or bouillon gelatin or agar. All developed in mineral solutions containing saccharose, lactose, glucose, mannite, glycerin, potassium succinate, potassium lactate, potassium citrate, ammonium bimalate, sodium butyrate, sodium hippurate, asparagin and peptone, but not in those containing potassium tartrate, ammonium tartrate, potassium acetate, and sodium formate.

Inoculations made with authentic cultures of *B. coli* (obtained from Calmette, Malvoz, and Van Ermengem) and with related forms, gave infections on potato tubers treated with 1 per cent soda solution. All afterwards became parasites it is said on untreated tubers. Experiments were also made with the following bacilli: Typhoid bacillus from Gand, Liège, and Lille, Gaertner's *Bacillus enteritidis*, Moorzele and van Ermengem's bacillus from the meat of calf, Friedländer's bacillus, Lambert and van Ermengem's bacillus from liver.

All these strongly resembled *B. coli* when cultivated in gelatin and on cooked potato, but showed different chemical capacities. *Bacillus fluorescens putidus*, *B. fluorescens liquefaciens* and a bacillus forming yellow colonies, isolated from rotten tomatoes were also used.

All of these when inoculated into normal potatoes gave negative results. When tubers treated with soda solution were used, however, all attacked the tissues, and from that time were able to live as real parasites on several varieties of tubers. The typhoid bacillus was more virulent than the strains of *B. coli*. A second passage of this organism through potato produced a pulpy layer 10 to 12 mm. thick after 24 hours in the thermostat at 35°. When again cultivated in gelatin, the various organisms had the same characteristics as in the original cultures.

In the course of these experiments Laurent observed a gummy disease of the tubers of *Cattleya mossiae* (orchid). He isolated from these in cultures on must of beer gelatin a short bacillus which became parasitic on potatoes, and which he identifies as a form of *B. coli*. The gummy disease he attributes to excess of nitrogenous fertilizers. The signs in the orchid are the softening of the tissues of the tubercles which become deep brown and then black.

Laurent also isolated a bacillus supposed to be the cause of black-rot in tomato fruits. This differed much from *B. coli*. It did not grow in mineral solutions. On potato it formed golden yellow colonies, and it did not liquefy bouillon gelatin.

In another case, *B. fluorescens liquefaciens* was isolated from badly rotted tomato plants, which, according to the grower, had been cultivated on ground dosed with enormous quantities of manure and liquid fertilizers.

One grower noticed that tomato plants cultivated on soil which had been exposed to the air by spading were healthy, while those set into the same house in soil left untouched all winter contracted the disease which had been present the previous year. This Laurent thinks is due to the fact that the bacilli living saprophytically on the roots retained their virulence in the one case, while in the other where the decomposition of the roots was more rapid, the organisms had lost their virulence.

The following properties of *Bacillus coli* as a parasite on potato are given: It did not develop at 10° to 12° nor above 40°. It did not attack the cellulose of filter paper, or that of cotton, the pith of elder, or the seeds of the date palm, even if glycerin was added to the culture to permit rapid development of the organism. Liquids obtained by diluting and then filtering the pulp of attacked tubers, even those very active on the tissues of potatoes, resulted in nothing, even after several days, when tried on the varieties of precipitated cellulose. Cultures in peptonized bouillon, heated for 5 minutes at 75° are sterilized. The bacillus did not show any spores.

The surfaces of his tubers were not sterilized before cutting, they were only washed and then cut with a flamed knife. No checks appear to have been kept, *i. e.*, each half was inoculated. If I did things in this way I should expect to have mixed cultures continuously. Possibly all these results were obtained with *Bacillus coli*, but of this I am somewhat sceptical. The inoculated things were kept generally in covered crystallizing dishes, or under bell-jars, containing sterilized water.

Jensen (1900) experimented along the same lines as Laurent but could not get the same results. He concluded, therefore, so far as he could draw conclusions from his experiments, that the true *Bacillus coli* is not able to attack raw potato tubers which have been made alkaline with NaOH. When *B. coli* made any growth at all it was confined to the thin surface layer which had been killed by the alkali. The living cells beneath had normally acid cell-sap. On most of the potatoes no growth was visible. He used 1 per cent and 2 per cent solutions of NaOH and the tubers were first thoroughly scrubbed and soaked for 2 hours in 2 per cent corrosive-sublimite water and then cut with a sterile knife and handled with sterile forceps. The results were quite otherwise when less care was taken to work under sterile conditions, *e. g.*, when the potatoes were cut with an unsterilized knife. Then after some days the surface was covered with an abundant bacterial flora, but pieces inoculated abundantly with *B. coli* bore no more bacteria than uninoculated pieces. In some cases these intruders attacked the sound parts of the potato with solution of the intercellular substance and gas formation, but in most cases only the part killed by the NaOH was attacked, it mattered not whether the tubers were inoculated with *B. coli* or were uninoculated.

In 1902, Lepoutre published a paper on the experimental transformation of saprophytic bacteria into plant parasites. The question before him was whether this transformation could be induced in other bacteria than those Laurent had experimented with.

He states that his experiments were carried on with three species, *B. fluorescens liquefaciens*, *B. mycoides*, and *B. mesentericus vulgatus*, but one can not be at all certain from anything in his paper that he really had these particular species under observation. His field for experiment was the same that Laurent used and was divided into five plots. Each year plot I received an excessive application of nitrogenous fertilizer; plot II, of potassium; plot III, of superphosphates; plot IV, of lime; and plot V of sodium chloride.

The bacteria studied were sowed on the surface of sections of potato or carrot placed in closed crystallizing dishes and kept in the thermostat at 30°.

The first attempt was made with an organism found in a decaying potato, and which in consequence had some initial virulence. His identification of it as *B. fluorescens* appears to have been a rather offhand one, to wit: "Une culture sur bouillon gélatiné m'assura que c'était bien cette espèce." A little of the infected pulp was placed on slices of carrot. On the third day some blackish glairy spots appeared on the sections of roots from plots I and V. The others were unaffected. The sections were then trimmed (refraîchies) and reinoculated, this time with the product of the previous inoculation on sections from plot I. After 2 days development had taken place only on sections from plots I and V, but the attack on the tissues was more pronounced than in the former passage, especially on the roots from plot I.

Turnips from the five plots were then inoculated with *B. fluorescens* obtained from the last passage upon carrots from plot I, and with *B. mycoides* and *B. mesentericus vulgatus* from bouillon cultures. The first of these showed itself the most active of the three. Roots from plot I were most predisposed to infection. These were attacked to a depth of 5 mm. The parenchyma was completely disintegrated and replaced by a very soft, decidedly alkaline pulp. The other two species caused only a slight attack on turnips 3 days after inoculation, the most being on turnips from plots I and IV.



A new series of turnips was then inoculated with the three species of bacteria obtained from the last experiment on plot I. After 24 hours *B. fluorens* had attacked the roots from plot I to a depth of 5 mm., and those from plots IV, V, II, and III, in the order named. The other two bacilli attacked the turnips of all plots in the same relative degree but much more feebly.

A third series of sowings on turnips gave within 24 hours a general attack to a depth of from 5 to 8 mm. After 2 days some sections 2 cm. in thickness were traversed through and through. A brownish liquid with fetid odor and alkaline reaction ran out upon the bottom of the crystallizing dish. [This statement relates probably to his *B. fluorens*.]

After three passages the three bacilli had thus become parasites on turnips, especially those in plots I and IV. *B. fluorens* was evidently the most virulent. The other two formed on the surface a sort of pellicle, which he says, by cutting off the air, no doubt stopped the progress of the invasion.

The following experiments were made on carrots. Slices of these roots, taken from the five plots, were sowed with *B. fluorens* from a previous culture on carrot, and with the other two species parasitic on the turnips of plot I. After 24 hours carrots from plots I, IV, and V were attacked, the last most feebly. Here again *B. fluorens* was the most active.

The passage of the other two bacilli of the turnip upon the carrot diminished their virulence. But a second series of inoculations, using the products of the attack of each species on the carrots of plot I, gave a general attack on carrots in all the plots. The alteration was deepest on the roots of plots I and IV, and most rapid with *B. fluorens*.

A third passage had communicated to the bacilli a strength of attack such that in 24 hours the carrots were decomposed to a depth of more than 5 mm. Subsequent passages further increased their virulence. That of *B. fluorens* was so great that slices 50 mm. in thickness were completely softened in a few days.

The products of disintegration of the tissues, and the brownish liquid proceeding from them, had a decidedly alkaline reaction. Moreover, the cultures of *B. fluorens* gave off a strong odor of ammonia.

To sum up, the carrots and turnips which were subjected to the influence of excessive applications of nitrogenous fertilizer or of lime showed least resistance to parasitic invasion. On the other hand, the use of phosphoric acid diminished the predisposition to infection.

These results are said to confirm those of Laurent obtained with *B. coli*.

Mature tubers of Jerusalem artichoke and roots of sugar beet appeared naturally immune toward the decay caused by the bacilli studied.

Jerusalem artichokes from all five plots were inoculated with *B. fluorens*, virulent on the carrot. After 4 days the tubers of plot IV only were feebly attacked and a second passage on the same medium did not increase the parasitic aptitude.

Attempts at inoculation on the sugar beet failed. By inoculation after exposure to 1 per cent soda solution the rot was obtained, but a second transfer to normal roots gave only a slight result, showing that the immunity in this case is very real.

On account of an accident the potatoes from the experiment field could not be used. Consequently fodder varieties cultivated in other fields were employed.

The pulp of turnips and carrots attacked by the three species of bacillus was used as inoculating material with negative results. An artificial means, invented by Laurent, was then used to diminish the natural immunity. This consisted in immersing the halves of potatoes for 1 hour in a 1 per cent solution of soda. Inoculation of these was followed, after 24 hours in the thermostat, by a destruction of the parenchyma to a depth of 3 to 5 mm. At the end of 2 days the attack of *B. fluorens* had penetrated to a depth of 15 mm. and that of the other two bacteria to a depth of 8 to 10 mm.

Normal potatoes, cut in two, were then sowed with the three species cultivated on the tubers plunged in soda. Beginning on the next day, *B. fluorens* produced a pulp 5 mm. thick. The other two sorts formed a sort of mycoderma as on the turnips and carrots. Subsequent passages on potato increased the virulence of the three species so that even the most resistant tubers were finally attacked. The author considers the parasitic aptitude of *B. fluorens* remarkable and thinks this species is undoubtedly a dangerous enemy of many cultivated plants.

In the pulp formed by *B. fluorens* the cells were completely disassociated, but the starch grains remained intact. The pulp infected by the other two bacilli was firm and lumpy, and many of the cells remained in contact.

The following observations were made on *B. fluorens*:

Inoculated potatoes or turnips examined in March showed plainly that while the pulp was alkaline, the tissue immediately underlying it was acid. In this acid zone no bacteria were present, yet the protoplasm was contracted and the cells were beginning to separate.

Juice pressed from infected turnips and filtered through a Chamberland bougie was brownish with an alkaline reaction. A part of this liquid was neutralized with dilute hydrochloric acid and

separated into three parts. To one part (A) was added 2 per cent oxalic acid, to the second (B) a little lime water, and the third part (C.) was heated to 62°C. for 5 minutes. A drop of essence of mustard was added to prevent invasion by bacteria. Small slices of carrot, turnip, and potato were plunged into these liquids. Two hours afterward the superficial tissues in A and B were disorganized and the protoplasm in the cells contracted. In C the tissues remained compact but the protoplasm was more decidedly contracted than in B.

Here are found the two agents described by Laurent, an enzyme which dissolves the middle lamellæ, and a substance which contracts the protoplasm. The former is destroyed at 62°C., and works best with *B. fluorescens* on acid media; the latter is resistant to a temperature of 62°.

Similar experiments were made with the juice from infected potatoes with similar results. An enzyme was present which dissolved the middle lamellæ in the presence of lactic and acetic acid accompanied by coagulation of the protoplasm. An alcoholic precipitate from the filtered extract of these cultures when dissolved in water caused disintegration of the membranes. The presence of acetic and lactic acids in the same liquid was revealed by analysis.

The protoplasm of potatoes is not contracted by a 1 per cent solution of these acids, though that of Jerusalem artichokes, carrots, and onions is thus affected. Such a concentration is probably not reached in cells at the limit of the contaminated tissues. Other products of secretion add their toxic action to those of the acids and determine the death of the cells before the penetration of the bacteria. Cellular separation due to the action of an enzyme must take place before the bacteria can penetrate the tissues. This enzyme appears to diffuse more slowly than the toxic substances. Alkalinity in the pulp containing bacteria is due to the products of the decomposition of nitrogenous materials contained in the cells. Thus ammonia is formed, which neutralizes the acids. Its presence is evident from the odor, and it may be liberated by distillation with potash.

The intervention of ammonia is necessary to prevent the toxic action of organic acids produced by the bacteria in the decomposition of sugars. Thus, he thinks, may be explained the pre-disposition of tubers from plot I, where nitrogenous fertilizer was used.

A nitrogenous alimentation provokes a more important assimilation of nitrogenous compounds, albuminoid substances, amides, or others, all very favorable to the nutrition of bacteria and to the production of residual ammoniacal compounds.

Recently M. Peterman has called attention to the great amount of non-albuminous nitrogenous material in tubers of potatoes subject to *Peronospora*, and a corresponding lack in resistant varieties. This is new evidence for the relations existing between the composition of plants, their alimentation, and the development of their parasites. This relation should be as true for mineral substances as for organic ones; for carbohydrates as for nitrogenous compounds.

The following is given as an example: Cultures of *B. fluorescens* made in April on potatoes were unsuccessful. The potatoes were immune. The same was true in May on diverse varieties. The organism seemed to have lost its virulence. In June, however, similar cultures on new potatoes gave positive results; the bacteria were again active, and several passages on potato restored their former virulence completely. This immunity in spring may be explained only by the exhaustion of reserve sugars by respiration and by the growth of shoots. *B. fluorescens*, incapable of attacking starch, is not able to produce from the old tubers the toxic substances which kill the parenchyma of the tuber. Here immunity results from impoverishment of the host.

At the suggestion, and under the guidance of Beyerineck, Dr. van Hall undertook a series of experiments to determine which of the saprophytic bacteria in the soil are able to cause decay in the subterranean part of plants, *i.e.*, are facultative parasites. In 1902, as a result, he published a paper on *Bacillus subtilis* and *Bacillus vulgatus* as plant parasites. The following is an abstract of this paper:

He placed in Petri dishes, on moist filter paper, freshly cut slices of different plants. Over some he poured a small amount of water in which soil had been shaken; others he streaked with damp soil. The soil used was taken from several localities. Decay did not occur in any case at room temperatures (23°, 30° C.), but was obtained very often at thermostat temperatures (37°, 42° C.). In every case, with one exception, decay was caused by one or other of two bacilli, identified as above. No others appeared when, according to Laurent's method, the slices were kept for an hour in 1 per cent solution of potassium hydroxide before inoculation, to reduce the acidity of the cell-sap.

*Bacillus subtilis* attacked Jerusalem artichokes, potatoes, and hazel nuts. After 24 hours in the thermostat at 37° C., slices of the artichoke and potato inoculated as above showed moist dark-colored spots on an otherwise dry white surface. These spots spread rapidly and after another 24 hours covered almost the entire surface. These spots swarmed with bacteria all, or almost all, of which belonged to *B. subtilis* as shown by colony cultures. The spots on the hazel nut were slimy but uncolored.

Cultures of *B. subtilis* were always similar in appearance when made from the decaying spots, but a peculiar variation developed in cultures after a time. Streak cultures on malt-agar often showed in 2 or 3 days transparent outgrowths, contrasting strongly with the milky-white or yellowish streak. A microscopic examination showed these transparent outgrowths to contain no spores, though spores were present in great numbers in the streak itself. By transfers pure cultures of this "asporogenous variety" were easily obtained and remained free of spores when further cultivated. Yet the ability to form spores was not, he thinks, entirely lost, for now and then atavistic forms arose which were spore-producing. Such forms were too rare to be detected by the microscope, but when a rather old culture was scraped from the agar, covered with water, heated to boiling and then poured over malt-agar or sterilized potato there frequently appeared one or more colonies which belonged to the original spore-forming variety. [It is not stated whether this was direct from the translucent part of a spore-bearing streak; a pure culture from a colony of the non-sporogenous form; or a subculture from one of the outgrowths, and this is important in judging whether he had two organisms on the start or only one.]

Inoculations were made with pure cultures of both forms (sporogenous and non-sporogenous) as follows:

The parts of the plants to be used were scrubbed with soap under the tap, then pared, and kept for a few minutes in 2 per cent solution of corrosive sublimate. Finally they were washed in sterile water. Freshly cut slices of this material were then placed in Petri dishes and streaks were made from pure cultures. Results showed that both sorts were equally active. At 30° C., potatoes, Jerusalem artichokes, early turnips (Mairübe), celery, and carrots were badly decayed, and kohlrabi, hazel nut and chestnut slightly attacked; while all of these, except the chestnut, were badly decayed at 37° C. Many more varieties of plants succumbed than had done when earth was strewn on the section. This indicated to him that the result of infection depends on the number of bacteria in the material used for inoculation. No results were obtained from inoculation on any of these plants when kept at 23° C.

Three months later repeated attempts to inoculate from these same pure cultures, kept during that time on artificial media, gave negative results, except in case of the potato which the sporogenous form still rotted readily and the non-sporogenous form slightly. Virulence was, therefore, greatly reduced by culture for 3 months on artificial media. This virulence was restored to both forms by a single passage through the potato.

Inoculations were then made on whole tubers of potato, Jerusalem artichoke, and the Mairübe (*B. rapa rapifera*). The surface was cleaned and freed from surface bacteria, as before mentioned, and the bacteria (sporogenous form) introduced through a small wound. The tubers were then placed in sterile glass vessels, in the thermostat and kept at constant temperatures (37°, 30°, 23° C.). At 37° C. it required only 4 or 5 days to complete the decay of each species. At 30° it required 10 to 12 days, while at 23° no result was obtained.

The decay is a characteristic soft rot, the progress of which may be easily followed. In the cells between the healthy and the decayed parts, the protoplasm contracts and becomes granular. At the same time the cells separate with the disintegration of the middle lamellæ. This is due, not to the presence of bacteria, they having not penetrated so far, but to their secretions. *B. subtilis* is not able to digest cellulose nor to penetrate into the cells, hence it is found only in the intercellular spaces. As destruction progresses the granular protoplasm disappears almost entirely while the starch granules, which remain intact longest, gradually lose their sharp outlines, mass together and slowly dissolve. In streaks on nutrient agar containing 0.5 starch, tests with iodine after 3 or 4 days growth showed absence of the blue color in the vicinity of the streak.

A characteristic browning or blackening accompanies this process in potatoes and Jerusalem artichokes. This is probably due not to the bacteria directly, but to the oxidation of an enzyme (tyrosinase), which the bacteria have not destroyed.

The decayed parts also gave off a rather characteristic odor, chiefly of trimethylamin and ammonia. The reaction was always alkaline.

In comment on the above it may be said if one formulated the following hypothesis, viz., van Hall was working with mixed cultures, a non-sporogenous parasite, and a spore-bearing saprophyte related to or identical with *Bacillus subtilis*, I do not see how it could be combated with any degree of certainty by means of any statements in his paper, since he nowhere speaks of beginning any of his successful inoculations with descendants of a single well-identified spore.

To demonstrate more clearly the presence of a virus secreted by the bacteria, potatoes decayed by *B. subtilis* were crushed and their juice filtered through a porcelain filter. One drop of this

filtrate placed on a slice of potato killed a large portion of it within 24 hours. When a voluminous precipitate, obtained by adding (2:1) alcohol, was dried in the thermostat, pulverized, and placed on potato, it was very destructive. After 2 hours at 37° C. much of the tissue was attacked and killed. Heating to the boiling point destroyed the toxic effect of the filtrate. At 37° it was very active, at 30° weak, and at 23° ineffective. Neutralization with hydrochloric acid did not destroy its toxic qualities. The presence of this toxic substance was also demonstrated in the artificial cultures.

Another experiment to demonstrate the secretion of a toxin was as follows: A small piece of malt-agar, on which a streak culture had made a luxuriant growth, was cut out with a sterile knife, placed on a freshly cut slice of potato, and kept in the thermostat at 37°. Within one day the effect of the toxin was visible, for just below the strip of agar the tissue was dead and formed a soft mass. Streak cultures on several other sorts of nutrient agar all grew luxuriantly, but were different in their toxic effects. Strips of agar from cultures on bouillon-agar acted as destructively as those from malt-agar, but the action of those from saccharose-pepton-agar, and saccharose-asparagin-agar was weak, while strips from cultures on saccharose-potassium-nitrate-agar, and saccharose-ammonium-sulphate-agar produced no toxic effects. Most of the paper is devoted to *B. subtilis*. *Bacillus vulgatus* required for parasitic activity a higher temperature than *B. subtilis*, attacking occasionally a few plants at 37°, but many at 42° C. Shiny spots were formed which were at times covered with a folded bacterial membrane. On potato at 37°C. there appeared occasionally among the spots due to *B. subtilis* small, round, viscid, slimy spheres, which decayed small portions of the tissue. These spots contained *B. vulgatus* almost exclusively. On agar cultures from these spots the bacteria showed some differences, but only minor ones, probably indicating sub-species.

*B. vulgatus* lost and regained its virulence in the same manner as *B. subtilis*. Decay progressed similarly, only the color and odor differed somewhat. A strong toxin was produced in cultures on all the media used.

The fact that these bacteria become parasitic only at high temperatures makes it improbable that they are ever responsible for damage in this climate (Holland), but it is not impossible that in warmer climates they might be dangerous agents of decay.

In 1903 Muth published an account of his experiments on the variations in seed-germination (made on 32 species), in which he states that his results lead him to disagree with Laurent, Lepoutre, and van Hall regarding the adaptability of *Bacillus coli*, etc., to a parasitic life. The experiment which led him to this conclusion is given below.

He tested the effect of inoculating before germination, carefully washed seeds with pure cultures of fungi (*Aspergillus*, *Penicillium*, *Mucor*, *Botrytis*, and *Cladosporium*), and of bacteria (*B. coli*, *B. mycoides*, *B. fluorescens liquefaciens*, *B. astroporus*, and a bacillus out of truffle conserves).

The fungi gave very positive results; almost all the seeds were attacked. The results with bacteria on the other hand were almost completely negative. The infections produced were doubtful ones, and so few in proportion to the whole number inoculated that he considered further experiments necessary to determine whether there was any action whatever.

## LITERATURE.

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## INCEPTION AND PROGRESS OF THE DISEASE.

### MANNER OF INFECTION.

Infection may take place in a number of ways either through natural openings or by way of wounds. These ways will now be considered, beginning with the most direct method.

### WOUND INFECTIONS.

The wounded surface, even though small, affords in case of many plant-organs a very suitable soil for the right sort of a bacterium. Here it first makes a little growth at the expense of the extruded cell-contents, *i. e.*, it multiplies first of all in the dead tissues of the wound. If the lodged organism is not a facultative parasite, growth in the wound either does not occur or ceases very soon, and no disease is induced. If, on the contrary, the organism is a wound-parasite, it does not remain confined to the original wound very long. In such cases, growth is more vigorous, and this presumably sets up osmotic changes determining a movement of the plant-juices toward the wound. A protective cork-layer is not formed under the wound, or is formed only imperfectly, and through the intercellular spaces and the neighboring vessels there is an open passage way into the depths of the tissues, a way which the parasite is not slow to make use of. Enzymes, toxins, acids and various by-products of the bacterial growth also undoubtedly play their part, weakening the cells of the host or destroying them outright. With increasing supplies of food, and a nidus rendered suitably alkaline by their own excretions, the bacteria multiply more and more, obstructing some tissues and dissolving, displacing, and crushing others. The tissues are poisoned more and more by absorption of the continually increasing quantity of bacterial by-products, cells are separated, cell-walls are softened or dissolved, protoplasm, amids, acids, starch, and sugars are consumed. Beginning, therefore, with a tiny superficial nidus in an open wound, a facultative parasite gradually burrows its way into the deeper tissues, forming closed cavities or open wounds, and finally destroying the entire plant or limiting its operations to special organs, as the case may be. Such is the impression one gets from a study of wound-infections.

The action of such a bacterium may be slow or rapid, depending on its own habits, on the degree of resistance or susceptibility of the host-plant, and finally on whether the surrounding conditions, such as temperature, light, food-supply, and water-supply are most favorable to the host-plant in its opposition or to the parasite in its attack.

The susceptibility to a given disease varies greatly in different races of the same plant, and also from individual to individual, if for convenience one may be allowed the use of this word in speaking of plants. In most cases the reason for this difference in susceptibility is unknown.

In my account of particular diseases I shall discuss fully the manner of infection, and desire here to make only a brief statement.

*Bacillus carotovorus* Jones and *B. aroidae* Townsend are very good examples of wound-parasites. We do not know that they ever enter the plant except through wounds. In dry tissues they make only a slow progress, but in juicy tissues, at suitable temperatures, they make an extremely rapid growth, and the destruction of the host is correspondingly great. A single needle-prick introducing either of these organisms into the fleshy tissues of a large green cucumber is sufficient to cause the whole interior to break down into a soft watery mass of disintegrated cells in the course of one or two weeks. The first organism, introduced

into a carrot-root in small numbers, under favorable conditions usually will rot the whole of it within a week. There are various other bacteria of this type, *e. g.*, Spieckermann's soft rot organism and *Bacillus phytophthorus* Appel. In general, they are omnivorous in their tendencies. *B. aroideae* is able to rot the fleshy parts of at least 13 plants belonging to half a dozen widely different families, and *B. carotovorus* has almost or quite as wide a range of activities. The green leafy parts of these same plants are attacked either not at all or less readily by these soft-rot organisms, for the rapid multiplication of which tissues full of water appear to be essential.

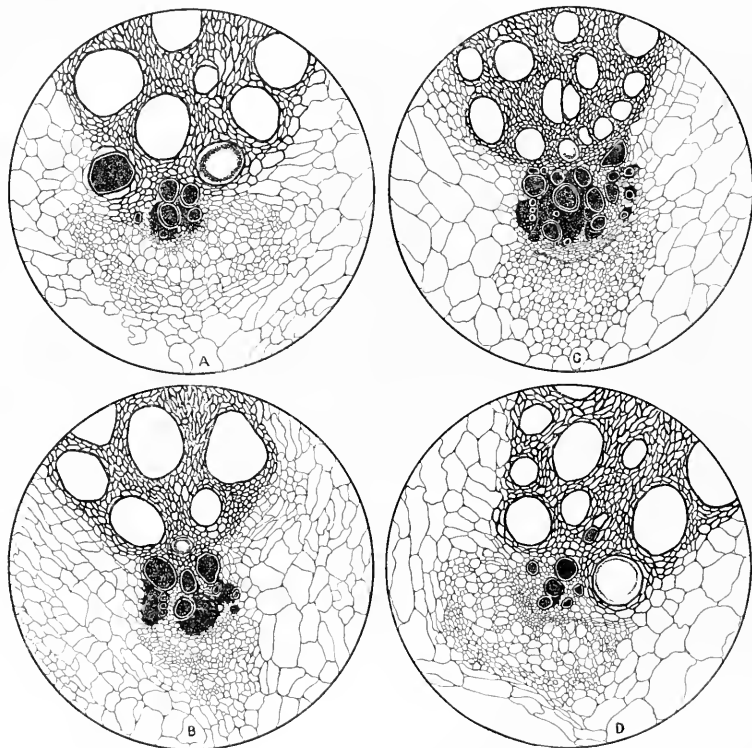


Fig. 6 \*

*Bacillus tracheiphilus*, the organism of the cucurbit-wilt, is a wound parasite of a somewhat higher type. It usually attacks the plant through its leaf-surface (plate 1), and it generally sticks pretty closely to special systems of tissues, especially in early stages of the disease. It is par excellence an occluder of the vascular system (fig. 6), in which it often extends for a distance of several feet from the original point of infection. It usually enters the plant through wounds made by leaf-eating insects (fig. 7). Whether it ever gains an entrance through natural openings is still a mooted question. The few experiments made by

\*Fig. 6.—Cross-sections of four inner bundles from a cucumber-stem in stage of wilt shown in plate 1, fig. 2, differentially stained to show bacterial masses confined to spiral vessels, and their vicinity, where cavities are beginning to form in non-lignified vessel parenchyma. A few pitted vessels are occupied. The phloem, most of the xylem, and all large-celled tissues between bundles and toward the periphery of the stem are free from bacteria.

the writer would seem to indicate that it does not enter through unbroken surfaces. Three of the four plants which were atomized thoroughly with a virulent culture did not contract the disease, and the fourth showed signs of it only after 20 days. One of the three which remained free bore aphides. More experiments should be made.

Injuries due to insects and other small animals, *e. g.*, nematodes, undoubtedly chiefly favor the entrance of bacteria, but I think we must also regard injuries due to frost and to

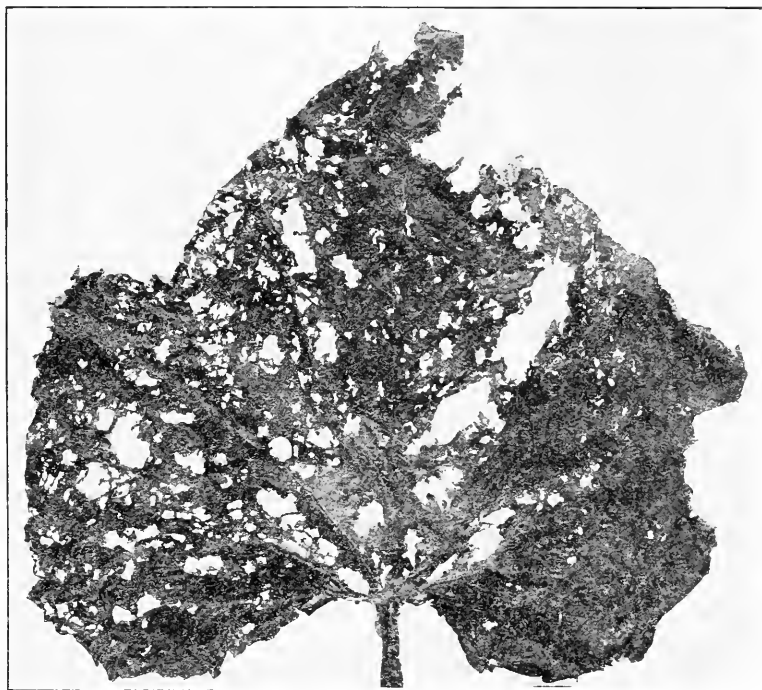


Fig. 7.\*

hail as among the predisposing causes in certain cases. Pure frost injuries when slight may also resemble bacterial leaf spots as in case of the tender magnolia shown in fig. 8.

Many bacterial plant-parasites are able, however, to enter the plant in the absence of visible wounds, and this brings us to the question of infection through natural openings. These bacteria we will now consider. They are a more interesting group than the strict wound-parasites for this reason, if no other, that they are able to begin business on a smaller capital.

\*Fig. 7.—Cucumber leaf wilted by *Bacillus tracheiphilus* and then gnawed by striped beetles *Diabrotica vittata*; introduced to show partiality of this beetle for diseased leaves. One other wilted leaf on this plant was gnawed in same way, while a dozen turgid leaves were scarcely touched. These two leaves were primary infections transmitted by *Diabrotica* from squash leaves which were wilting as a result of my pure culture needle-puncture inoculations. The presence of the bacteria in great numbers in several different lobes of this leaf was demonstrated microscopically after photographing.

## INFECTION THROUGH NATURAL OPENINGS.

Inasmuch as certain scientific men of excellent reputation have doubted the possibility of any spread of bacteria through living plants except by wound-infections, it appears to be worth while to summarize somewhat carefully what is known of bacterial infections in the absence of visible wounds. The greater portion of the plant-body in the higher plants is well protected by a cutinized epidermis or by a still more resistant cork-layer, through which bacteria would find it difficult to make their way. There are, however, many natural openings, wholly unprotected places, and it is through these that infection takes place.

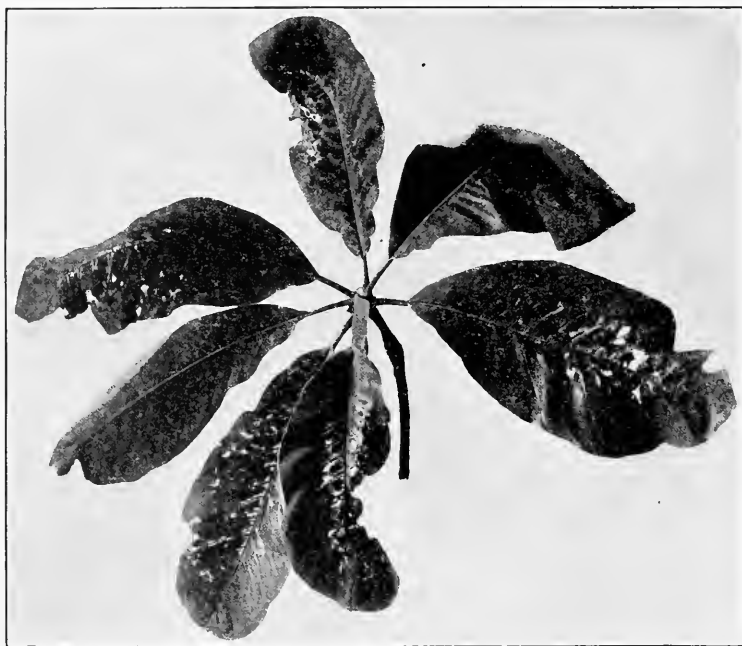


Fig. 8.\*

*Nectarial infection.*—The best-known case of infection through the nectaries is that of the pear, which is commonly attacked in this manner by *Bacillus amylovorus*. The organism, brought to the plant by bees and other nectar-sipping insects, multiplies enormously in the floral nectaries, which are blackened and killed. From this nidus the blight bacillus passes into the ovary and down the pedicel of the flower into the stem, which blights in turn. In moist, warm springs the progress of the floral infection is rapid, and it is not at all uncommon to find thousands of blighted blossom-clusters in a single large orchard. The organism attacks vigorous young shoots in the absence of blossoms, but infection by way of the nectaries is extremely common. Blossom-blight was observed by Dr. Arthur, who called attention to it in several publications, now 20 years old, but he does not appear to

\*Fig. 8.—Twig of *Magnolia fraseri*, showing frost injuries on five immature leaves; other two leaves developed after frost and are free from injury. Grounds of U. S. Dept. of Agriculture, May 2, 1903. The light frost occurred in April and none of the leaves showed the usual aspect of frosting.



have studied this phase of the subject experimentally. In 1891, Waite sprayed pure cultures of *Bacillus amylovorus* upon pear-flowers and obtained many cases of blossom-blight. This was studied in all stages, from the first incipient multiplication of the bacteria in the nectar to the destruction of the flower and the passage of the bacteria down the pedicel into the stem. By protecting the flowers from the visits of insects by means of mosquito-netting, this artificially induced blossom-blight was restricted to certain branches. This particular experiment was made in an orchard in Kent County, Maryland, which was remarkably free from natural blight and had been for years. In other experiments, not in that orchard, Mr. Waite again produced blossom-blight on certain clusters of pear-blossoms by infecting the floral nectaries and by allowing the bees to have free access to these blossoms he succeeded through their agency in transmitting blight to other flower-clusters on the same tree. One of these experiments took place on the grounds of the United States Department of Agriculture, an isolated tree previously free from blight being used for this purpose. Bees were observed to visit the infected flowers and, subsequently, flowers on other clusters, which flowers afterwards blighted. Some of these bees were caught, their mouth parts excised, and cultures made therefrom by means of poured-plates in Petri dishes. Colonies obtained in this way closely resembled the pear-blight organism, and inoculations therefrom produced the disease in sound pear-shoots, thus demonstrating beyond dispute the actual presence of the pear-blight organism on the mouth parts of the suspected bees.

Everybody connected with the plant pathological work of the U. S. Department of Agriculture at that time had knowledge of these results. The writer, among others, saw all of the experiments described and knows that they were well done and that the above brief outline can be accepted as an accurate statement of what actually took place.

In 1898 the writer produced Wakker's yellow disease of hyacinths on two occasions by inoculating through the flowers, but not all of the inoculated plants contracted the disease, and nothing is known respecting the natural occurrence of this disease as a result of nectarial infection. The same year a soft white rot of hyacinths, of the same type as Heinz's rot, was observed by the writer to originate in particular flowers and end in the destruction of the plants. It is deemed probable, therefore, that in the field both of these diseases may sometimes begin in the floral nectaries and be distributed by nectar-sipping insects. The hyacinth gardens of Holland, where these diseases occur naturally, will afford a final answer to this question.



Fig. 9.\*

\*FIG. 9.—Marginal leaf-infections on cabbage (No. 400) obtained by atomizing on a pure culture of *Bacterium campestris*, shaken up with sterile water. Inoculated Dec. 9, 1904. *a*, photographed Jan. 6, with transmitted light, marginal halation being avoided by a close fitting paper screen which cuts out leaf-serratures. *b*, contact print made Jan. 5 (lights and darks reversed). This is one of the plants that furnished material for the drawings shown in vol. I.

*Water-pore Infections.*—Early in 1897, by plunging healthy leaves into water containing the bacteria, the writer obtained numerous infections of the cabbage by way of the water-pores using *Bacterium campestre*, and later drew attention specifically to the whole subject of stomatal infection (September 1907), which had hitherto belonged only to the domain of speculation. A little later, as a result of field observations, he pointed out (January 1898), that a large proportion of the natural infections in cabbage and similar plants takes place through the water-pores, which are groups of modified stomata situated on the leaf-serratures. These field observations were made in Michigan, Wisconsin, Ohio, and Western New York. They covered a period of several weeks of active work in cabbage-

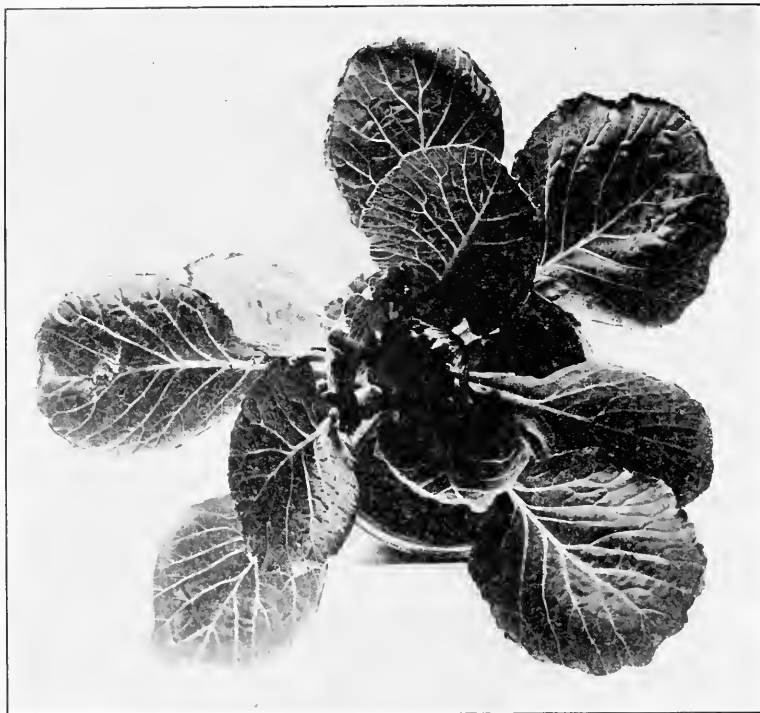


Fig. 10.\*

plantations aggregating hundreds of acres, and during this time the writer saw thousands of plants in all stages of the disease. These statements respecting infection by way of the water-pores were disputed in 1899, by Dr. Fischer on *a priori* grounds and were still held by him to be doubtful in 1903, but on Long Island, where this disease prevails extensively, the writer had opportunity for additional observations in 1902 and sees no reason for changing his statements in any way. He also obtained the disease in 1904, through water-pore infections of the cabbage by spraying upon the plants young agar cultures of *Bacterium*

\*FIG. 10.—Cabbage-plant No. 400 inoculated Dec. 9, 1904, by spraying. Photographed Jan. 11. Central leaves have grown since inoculation and are free from infection, while nearly entire margin of older leaves is destroyed as a result of water-pore infections. This plant belongs to the same series as that shown on plate 2. For details from the margin of one of these leaves, see fig. 9.

*campestris* diffused in sterile water (plate 2, and figs. 9, 10). The infection of the black rot takes place through water-pores of the cabbage, turnip, mustard and various other plants of the family Cruciferae, and the bacteria may be traced very readily from the substomatic chamber down into the vascular system of the leaf until they disappear, and have been so traced by the writer in a number of instances in serial sections made from properly fixed and suitably infiltrated material (see vol. I, figs. 76 to 79 and 115 to 117). The infection of cabbage plants through the water-pores was confirmed by Russell and Harding in 1898, and was also obtained in kohlrabi by Hecke in 1902, and more recently in cabbage by Brenner, one of Dr. Fischer's special students. As the writer stated in January 1898 (*Farmers' Bulletin*), the separate water-pore infections on a single large plant occasionally number several hundred, while very frequently the disease with its conspicuous black venation may be seen extending into the leaves of the plant from fifty or more leaf-serratures.

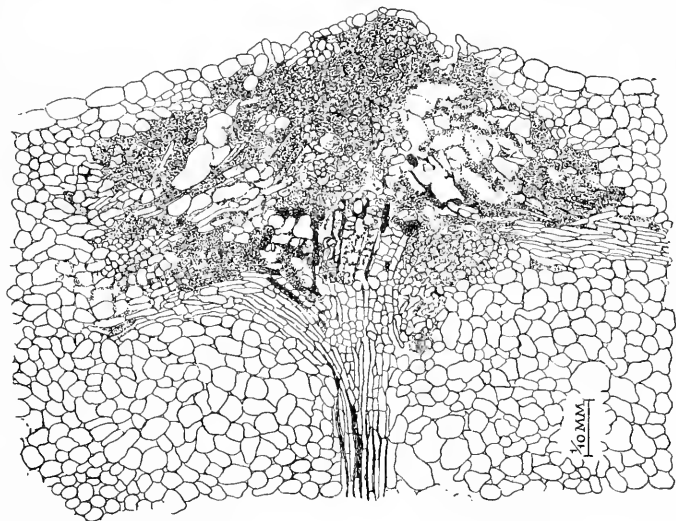


Fig. 11.\*

If the bacterial black rot is at all prevalent in a field, infections of this sort are as common and as easily observed as the plants themselves, and the bacteria may be demonstrated in abundance in sections made through any of the distinctly blackened leaf-serratures, and in a certain proportion of them before any stain is visible. For a time the bacteria are restricted to the tissues immediately under the hydrotodes and over the terminal bundles, but after a few days or weeks they form a closed cavity in the heart of the leaf-tooth (fig. 11) and make their way into the spiral vessels under the epithem. Once established in the vascular system, they multiply with great rapidity, and their downward movement through the vessels of the leaf and into the main axis of the plant is then often only a matter of an additional week or two.

*Stomatal infections.*—I will begin with the recently discovered black spot of the plum, to which I have thrice before called attention, namely, December 1902, December 1903, and December 1904, at meetings of the Society for Plant Morphology and Physiology

\*FIG. 11.—Water-pore infection of cabbage by *Bact. campestris*. The section is parallel to the surface of the leaf and passes through region of blackened leaf-tooth, the dotted part being that occupied by the bacteria. Collected at Jamaica, Long Island, N. Y., July 16, 1902. Slide 226 E7.

(notices in Science). This disease offers an excellent example of a natural infection, in which insects and other wound-makers play no part except possibly as common carriers, of which there is as yet no evidence. I did, indeed, suspect when the first specimens were sent to me in 1901 that this disease might be spread by the punctures of insects. This was owing to certain little cracks discovered in the center of some of the spots. There was no distinct evidence, however, of insect injuries except certain curculio stings, which had healed over and were sound. Later, when I visited the orchard and had excellent opportunities for studying the disease on thousands of plums, this hypothesis had to be abandoned as wholly untenable, the little cracks being found to be due to entirely different causes. Moreover, numerous serial sections which have been made in my laboratory show clearly that the disease does not begin in wounds. The earliest stage is simply a bacterial

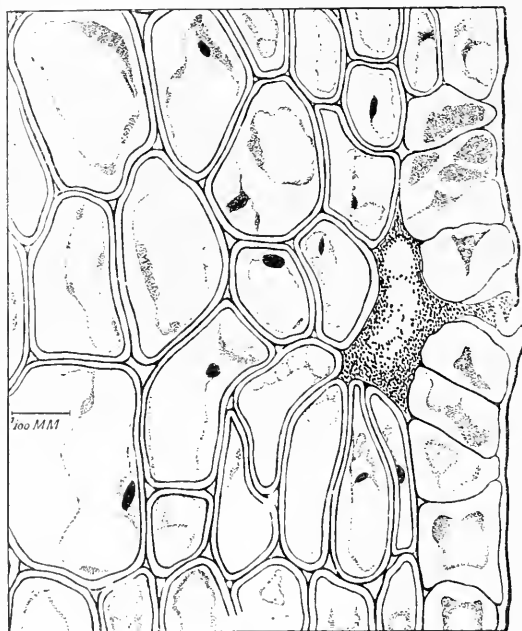


Fig 12.\*

occupation of the substomatic chamber (fig. 12). A little later a few neighboring cells are involved, and then we have the condition shown in vol. I, fig. 70. This stage precedes the appearance of spots, but with the gradual multiplication of the bacteria deeper tissues are involved, a small closed cavity filled with bacteria is formed, and the disease manifests itself externally by a minute water-soaked spot surrounding a single stoma and best seen by the use of a hand lens magnifying 8 or 10 times (plate 3, fig. 1). In this stage the epidermis is uninjured and on the fruit the spot bulges slightly from pressure of the growing bacterial mass underneath. A little later we have the bacteria escaping to the surface through the stoma or through a slight central rupture as shown in plate 4 and vol. I, fig. 72. Gradually the bacteria burrow deeper and especially wider, the spot enlarges, becomes black and sunken, and the bacteria

\*FIG 12.—Black spot on green Japanese plum. Earliest stage of infection by *Bacterium pruni*; bacteria confined to sub-stomatic chamber. A pure-culture infection, twelfth day; from the Takoma Park tree. Slide 308 C16, 2d section from right, middle row. Drawn with a Zeiss 3 mm. apochromatic 1.40 n. a. objective, No. 12 ocular and Abbe camera.

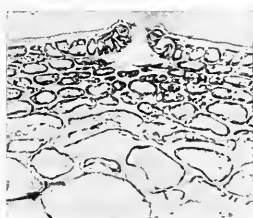
At a focus a little lower down, i. e., deeper in this section, the bacteria fill the whole sub-stomatic chamber. In sections to either side of this one the bacteria are limited, as here shown. Extremely fine dots within cells represent protoplasmic masses and not bacteria; the rounded and spindle-form, large, dark masses are nuclei.



Black Rot of Crucifers.

Cabbage-leaf (plant 402), showing three marginal infections which began in groups of water-pores as the result of spraying *Bacterium campestris* upon the plant. The spraying was made Dec. 9, 1904, and the photograph at the end of two months (Feb. 6).

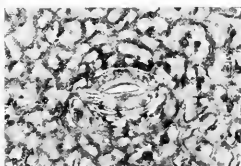




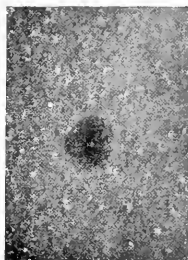
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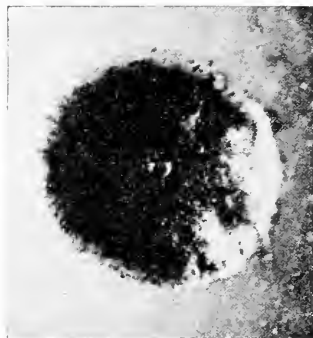
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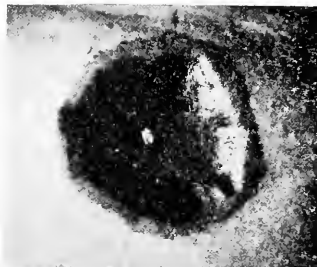
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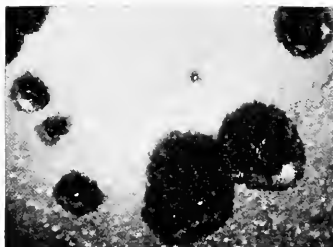
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#### Black Spot of the Plum.

- (1) Surface of a green plum (x 10), showing a white speck (stoma) in center of a very small spot, due to *Bact. pruni*. This is the earliest stage of the disease clearly visible to naked eye. Each of the numerous white specks has a single stoma in its center.
- (2) One of the tiny white specks more highly magnified so as to show the central stoma. x 200 (?).
- (3 and 4) Two sections through a normal stoma on the green fruit, showing empty sub-stomatic chamber, one passes through the center. x 200 (?).
- (5) A group of small spots on a green Hale plum, each of the smaller ones showing clearly a stoma in its center. x 10 (?).
- (6 and 7) Small spots (x 10) showing bacterial exudate. Spots further advanced than in fig. 1. In fig. 6, bacteria were issuing from 30 or 40 stomata, but, as in fig. 7, the central drop is larger. The reason for this is apparent at once on cross-section (see plate 5).





enlarging unaffected parts of the green fruits. The only conclusion I could come to, therefore, from extensive field observation made during 1902 and 1903, and from a careful study of serial sections made through many young spots, was that infection invariably takes place through the ordinary stomata, favored by the presence of rain drops or dew. Opportunity to test the validity of these conclusions by actual inoculation experiments did not occur for some time. During the first two years no trees were available. In 1903 trees were obtained, but through neglect to make transfers at the right time (many other lines of work being conducted simultaneously) the cultures were allowed to die, the only organism remaining alive when the cultures were needed being a greenish-yellow one, which was applied to the trees freely, but which proved to be destitute of pathogenic properties. One other set of experiments miscarried by reason of leaf-miners.

In the summer of 1903 cultures of the right organism were obtained once more from the Michigan orchard where the disease prevails every year, and in the summer of 1904 a thorough test was made, great numbers of stomatal infections being obtained both on leaves and green fruits by simply placing cultures of the organism in sterile water and atomizing this upon the tree. A Japanese plum tree about 5 years old and of the variety known as Abundance was selected for this experiment. The tree was about 12 feet high, with a corresponding spread of branches. It was very leafy and full of green fruits about one-third grown. The leaves and fruits were very smooth and free from gnawings of insects or fungous injuries, the only injury on the fruits being an occasional healed (corked out) curculio puncture. The tree stood in a garden at Takoma Park, a suburb of Washington, where no such disease was ever known before. No wounds were made, but the organism was scraped from several young slant-agar cultures into several hundred cubic centimeters of sterile water, and a portion of this was sprayed upon the tree by means of the apparatus shown in vol. I, figs. 92 and 93. The sprayings were made on the afternoon and evening of June 1, during a light drizzling rain, moist cloudy weather being selected for the experiment so that conditions might be as near as possible like those which frequently occur in Michigan during the period when most of the natural infections take place. The weather continued cloudy and moist for many hours, after which it was clear and favorable for the trees, with drouth at no time. For a week after the spraying there were no visible results. On June 8, on a few plums, the writer thought he detected some incipient spots, but the signs were very obscure even under the hand-lens and serial sections through the particular suspected tissues showed them to be free from bacteria. Distinct spots, yielding bacteria in abundance on sectioning, were visible, however, on many of the leaves and green fruits on June 14. It is probable, therefore, that they might have been detected in some cases as early as the tenth or twelfth day, but not much earlier, since the largest spots were still quite small. From this time on, the bacterial spots became numerous on both leaves and fruits and passed through their customary changes in an entirely typical way, so that when spotted plums were sent on from the Michigan orchard in July and were compared with those obtained by the spraying, they could not be distinguished (fig. 13). The organism was plated out of a dozen or more spots by several of my assistants, the poured-plates yielding in great abundance colonies of what was sprayed upon the tree. When made from young spots these plates generally contained pure cultures, but sometimes there were a few contaminating colonies of various sorts. When made from old cracked-open spots, the number and variety of contaminating organisms was greater and then often included fungi. Moreover, serial sections made from many spots yielded the same results as the similar sections already described as made from plums obtained from the orchard in Michigan, *to wit*: In early stages constant presence of the bacteria and absence of injuries due to fungi or insects. The writer has slides of sections cut from these sprayed plums showing all stages of the disease, from simple occupation of the substomatic chamber to the formation of extensive closed bacterial cavities with large destruction of tissues. These experiments show also

that not every chance organism will produce this disease when introduced into the stomata, but only *Bact. pruni*.

In 1898 the writer pointed out that *Bacterium stewartii* probably enters the plant through the water-pores situated at the tips of the leaves. The examination of diseased maize-plants found in southwestern Michigan led to this assumption. The tips of many of the leaves were dead, while the basal parts were living. The vessels in the tips of the leaves



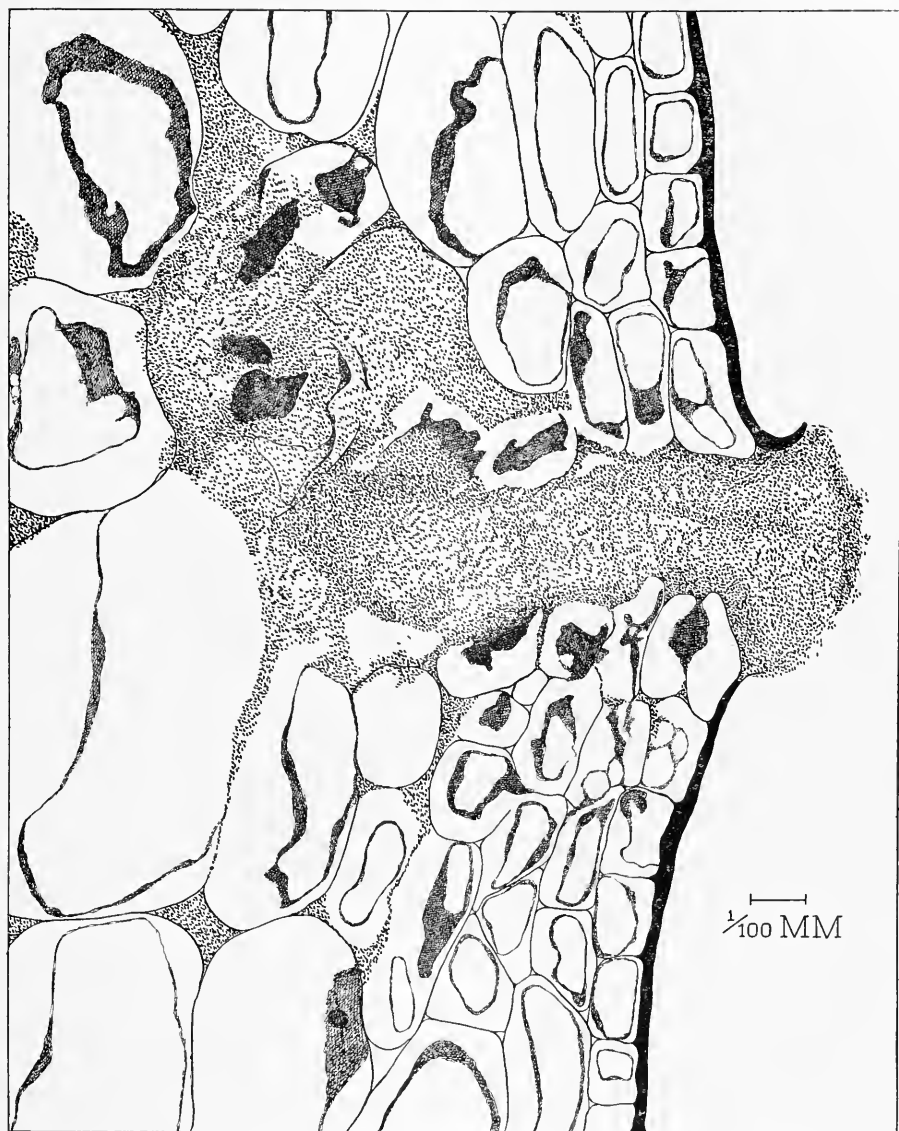
Fig 13.\*

were occupied by the bacteria, which could be traced downward through the bundles sometimes for a distance of one-third to one-half the length of the leaf and then disappeared. This pointed clearly to apical infection. The rest was inference. Since that publication, the writer has obtained numerous typical cases of the sweet-corn disease in Washington (where the disease was not then known to occur naturally) by stomatic or water-pore infections, one or both. Inasmuch as the sections thus far cut indicate infection by way of

\*FIG. 13.—Bacterial black spot of the plum:

Left: Pure culture inoculations. Plums from the sprayed tree of Abundance variety at Takoma Park, Maryland. Photographed July 23, 1904, spots 53 days old.

Right: Natural infections. Hale plums from the orchard at Duplain in Central Michigan.



Bacterium pruni.

Vertical section through a small spot on a green plum 14 days from date of infection, *i. e.*, before tissues have begun to collapse, showing tissue lifted, stoma disrupted, and bacteria oozing to surface. One of the sprayed plums at Takoma Park, Md. Slide 308 E 19. For an earlier stage of infection, see vol I, fig. 70.



the ordinary stomata (vol. I, figs. 74 and 75), the subject will be treated under this head, although it is considered probable that the other form of infection also occurs. In any event, the copious functioning of the water-pores must contribute very materially to the certainty of stomatal infection.

In 1902 the writer studied this disease on Long Island, where it is prevalent, and brought back pure cultures, the earlier ones received from Mr. Stewart having been allowed to die. The infections were obtained with various subcultures made from these original cultures. The plants were inoculated in the seedling-stage, the material for infection being obtained from cultures on slant agar. The inoculations were made by placing a small quantity of the bacterial slime on the tips of sweet-corn leaves which were extruding drops of water (vol. I, fig. 73). This was done in the afternoon, generally toward sunset, the plants, which were in small pots, being well watered and placed under the greenhouse bench to protect from the bactericidal action of light. After a day or two they were taken out of the shade and placed on the bench. They grew rapidly, and after some weeks, during which time they were repotted once or twice, they were planted out on one of the Department of Agriculture farms. They were well cultivated, experienced no setback by being transplanted to the open, and those which were not dwarfed by the early appearance of the disease grew satisfactorily. The first signs of the disease were at the tips of the inoculated leaves, and on some of the plants they appeared within a week. The first cases—that is, plants showing secondary or general signs appeared in about 3 weeks, but not many developed so soon, most appearing after 9 weeks. Cases to the number of several hundred continued to appear until frost put an end to the experiment about 3 months from the time of planting. These plants were several feet high when the general or constitutional signs first appeared. A macroscopic examination of all of these cases and a microscopic examination of a considerable number of them showed that the first parts of the stem to be infected were the basal nodes, *i. e.*, those which gave rise to the inoculated leaves. The organism finally occupied the vascular system quite fully, filling the bundles in many cases from the base of the stem to the male inflorescence, a distance of 3 to 4 feet, and also passing out into the bundles of the large middle and upper leaves, but usually not reaching the surface of any part of the plant, so far as observed, except on the inner surface of certain leaf-sheaths and on some of the inner husks of the ears (fig. 14).

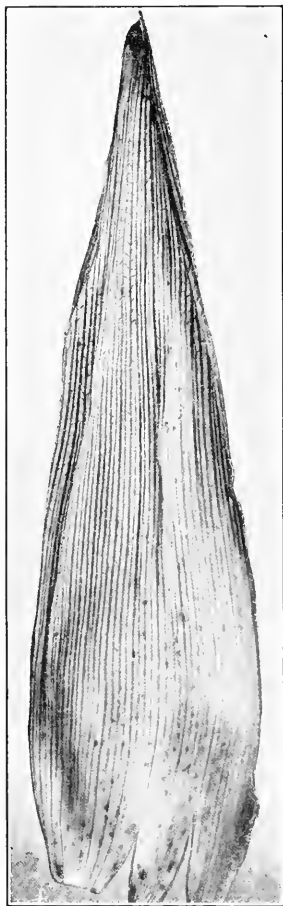


Fig. 14.\*

The spot disease of *Delphinium* (vol. I, fig. 127) is another malady in which infection takes place readily through the unbroken leaf-surface and stem-surface, *i. e.*, through

\*Fig. 14.—Inner husk of sweet corn, showing yellow spots and water-soaked areas in parenchyma, due to *Bacterium Stewartii*. In places also bacteria were oozing to inner surface. The bundles were occupied by the bacteria. From U. S. Dept. of Agriculture farm on the Flats below the Washington monument. Plant inoculated in seedling stage. Photographed Oct. 21, 1902. Movement of organisms was from young leaves downward into base of stem and thence slowly upward through vascular bundles of stem into ear, over 2½ months meanwhile having elapsed. Natural size.

stomata. The disease has been obtained a number of times during the last seven years by placing the bacteria in water and spraying this upon the plants. The leaf-serratures also blacken in this disease, and here infection probably occurs through the groups of water pores situated on their apex.

The genuine bacterial spot of carnations is a fourth disease of this type. It was produced a number of times by Lloyd Tenny, one of my assistants, who kept the plants moist under bell-jars for a day or two so as to get a deposit of water drops on the foliage, and then sprayed upon the plants sterile water inoculated with pure cultures of the organism. Infections are visible within a few days. They always begin in the substomatic chamber, and Petri-dish poured plates made from the interior of the spots on several different occasions have yielded pure cultures of the parasite, which when reinoculated by spraying has again produced the disease.\*

The spot disease of beans, caused by *Bacterium phaseoli*, is another example of stomatal infection. Serial sections through very young spots demonstrated this to me beyond reasonable doubt (fig. 15). Moreover, the disease was subsequently produced experimentally

under my direction by Deane B. Swingle. The spots appeared in large numbers in about 6 days as the result of spraying experiments and the earliest bacterial nidus was in the substomatic chamber. This manner of entrance explains, I believe, the fact once observed by Halsted that nine-tenths of the spots in this disease were on the western side of the pods—that is, as I interpret the phenomenon, on those parts where rain drops or dew drops would persist longest and thus give most opportunity for infection. The writer has observed the same thing in connection with the black spot of the plum.

The spot disease of broom-corn also arises by stomatal infection and has been so produced by the writer, using pure

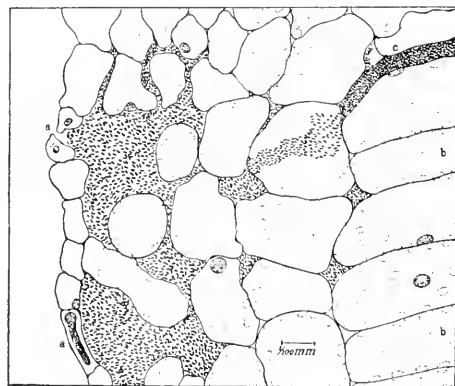


Fig. 15.†

cultures but not of *Bacillus sorghi*. For early stages see figs. 16 and 17.

The angular leaf-spot of cotton (vol. I, fig. 80) and the brown spot of *Pelargonium* are two other bacterial diseases in which infection commonly begins in the substomatic chamber. The writer has studied both of these diseases in serial sections and has reproduced the first by simply spraying the bacteria upon the plants (fig. 18). The *Pelargonium* leaf-spot was also so reproduced in 1906 by John R. Johnston, one of the assistants in my laboratory, using pure cultures.

\*In honor of Albert F. Woods, the organism here in question, which is entirely distinct from *Bacterium dianthi*, may be known as *Bacterium woodsii* n. sp. It is motile and non-sporiferous. It occurs as a short rod, single, paired, or united into small clumps. Its growth is pearly white on potato and on agar, forming circular, small surface colonies and spindle-shaped buried colonies on the latter. It blues litmus milk without separation of the casein (2 weeks). It is non-liquefying and non-reducing (nitrates). Its maximum temperature for growth is about 35° C. It grows well in beef-bouillon, potato-broth, and peptonized Uchinsky's solution, but not in Kauffin's fluid.

In early stages the spots somewhat resemble stigmose but become milky as they enlarge. At first the spots are water-soaked, then brown and sunken, somewhat resembling spots due to *Septoria dianthi*. As they enlarge the spots are usually surrounded by a narrow water-soaked border.

Habitat: Leaves, stems, and sepals of *Dianthus caryophyllus*, causing a spot disease.

†Fig. 15.—Cross-section of a bean-leaf attacked by *Bacterium phaseoli*. Natural infection, leaf collected in New Jersey. aa, stomata; one cut cross-wise, other length-wise. bb, uninjured palisade tissue, chloroplasts shown in outline only. The leaf has not yet entered into spot-stage nor have bacteria penetrated cells except perhaps at c, which is heavily stained and appears to be a shriveled palisade cell occupied by bacteria. The infection undoubtedly took place through the stomata. Slide 313 E1. Drawn with a Zeiss 3 mm. apochromatic 1.40 n. a., No. 12 compensating ocular and Abbe camera.

Recently, in the writer's laboratory, a bacterial leaf-spot of the cauliflower has been studied by Lucia McCulloch, and has been reproduced very readily by spraying upon healthy plants pure cultures of *Bacterium maculicolum* suspended in water, and here also infections were through the stomata as shown by serial sections.

In 1901, Zimmerman described a tubercular or knot disease of the leaves of certain Rubiaceous plants in Java, and figured a bacterial focus in the center of each little tubercle. In the earliest stage of the disease, before any tubercle had developed, he found very small nests of bacteria under certain stomata. It does not appear that he cultivated out any of these organisms or reproduced the disease by inoculations, but his figures are good and it is not likely that he was mistaken in the interpretation of his facts, or that the objects figured as bacteria should be anything but bacteria. Further work needs, however, to be done upon this disease.

Pierce's bacterial disease of walnuts and the olive-tubercle are two additional diseases which should be studied with reference to the possibility of infection taking place through the ordinary stomata. I believe it may, especially in the former. In fact it is difficult to explain the numerous spots on leaves, stems, and green fruits, on any other hypothesis. Pierce has reached the same conclusion; at least he has said that infection takes place readily in the absence of wounds. One spraying experiment made by the writer on a young olive shoot resulted negatively, but more should be made to permit of positive statements either way.

Several other cases are known to the writer where bacteria enter the plant and disorganize it through the water-pores or through the ordinary stomata, but enough has been said to call general attention to the fact, which is all that it was desired to do in this place.

Here, then, are a dozen well-established bacterial diseases, the organisms causing which are able to enter and attack the plants by way of the substomatic chamber in the absence of wounds and with only such moisture conditions as occur very frequently in nature. There are probably many other cases of this sort, and now that general attention has been called to the subject stomatal infections will probably be found to occur right and left, although it was maintained by Dr. Fischer, in 1897 and 1899, that not only has such a method of infection never been made out, but from the very nature of the case never could take place (see page 15).

*Lenticellate infection.*—When the epidermis of a plant gives place to a denser protective layer (the cork) stomata disappear, and more or less imperfectly closed openings, known as lenticels or cortical pores, take their place as aerating organs and are very conspicuous in some plants. During some portions of the year, in certain plants, the lenticels form open

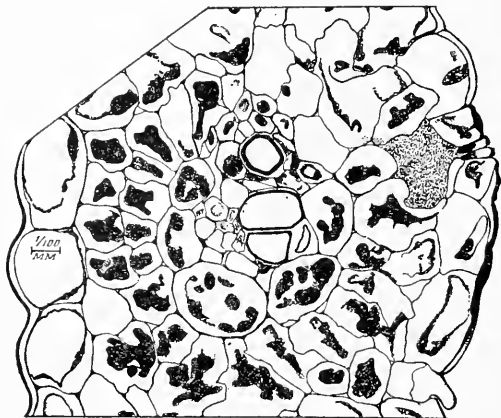


Fig. 16.\*

\*FIG. 16.—Stomatal infection of a broomcorn leaf. Substomatic chamber full, but bacteria not yet occupying intercellular spaces to any great extent nor any of the cells. Upper part of the bundle somewhat abnormal, possibly a branch arising. Slide 412 A11, left-hand section, fourth row. Drawn with a Zeiss 8 mm. apochromatic objective, No. 12 compensating ocular and Abbe camera. Paraffin section stained with carbol fuchsin. For later stage see fig. 17. Note: The organism here in question may be known as *Bact. andropogoni* n. sp. It is non-sporiferous, polar-flagellate (1-3), and white on culture media, forming small circular colonies on agar-poured plates. It is aerobic, non-liquefying, non-reducing (nitrates). For later stages of the disease see vol. 1, plate 20.

passage-ways into the deeper tissues of the plant, and it is probable that bacteria frequently make use of them in canker-diseases and the like, but of this we have, as yet, no clear proof. In case of potato-tubers, the lenticels swell and rupture when the earth is unduly moist or when they are kept in a saturated atmosphere, and an easy entrance is then afforded

to all sorts of soil organisms, especially to certain bacteria which induce soft rots. The writer has occasionally seen on the potato-tuber a small superficial bacterial rot-spot centered in a single lenticel. Sorauer observed this lenticellate infection many years ago. It was also noted by Reinke and Berthold in 1879, and has been seen by other persons. The writer first observed it in the laboratory in 1886-87, during a winter spent on diseases of the potato, and has seen it in the field in wet autumns. The earliest record of any sort of lenticellate infection appears to be that of Hermann Schacht. In 1856 he stated that scab often begins in the lenticels of the potato-tuber (*loc. cit.*, pp. 24 to 25, and his plate VII, fig. 3), and early the following year Caspary also called attention to the subject (*Botanische Zeitung*, 1857, column 116).



Fig. 17.\*

In 1907 Dr. F. C. von Faber described a bacterial scab of beets which begins in the lenticels. This was common in Germany in 1906.

Bacteria which have multiplied enormously in the interior of shoots may again reach the surface of the plants through lenticels as in case of the mulberry blight (fig. 3).

*Extrafloral nectaries.—The stigma.* No bacterial diseases are yet known in which infection takes place through extrafloral nectaries or through the stigma, but these are also unprotected places and such channels of infection are likely to be discovered if searched for.

#### PERIOD OF INCUBATION.

By this we mean the time between exposure to the cause of the disease and the first appearance of physical signs of disease. In plants the period of incubation is quite as variable as in animals. It depends on many factors, *e. g.*, the nature of the organism, its food requirements, its susceptibility to plant acids and the ease with which it produces ammonia or trimethylamin to neutralize these acids, its temperature requirements, the age of the cultures used, the volume of infectious material, the age of the plant, the rapidity of its growth, the juiciness of the parts, and, finally, individual or varietal resistance due to various unknown causes.

\*Fig. 17.—Cross-section of a leaf of broom-corn, showing a later stage of stomatal infection than fig. 16, but leaf not yet collapsed and endodermal cells *ee* not yet shriveled. Bacteria fill substomatal chamber and lie over and between cells but not inside of any. Tissues shrunken somewhat by strong alcohol. Two stomata *ss* through which bacteria entered, as indicated by sections to either side in the series. Xylem and phloem free from infection. In upper part of section bacteria lie over (*on*) three cells, sharply delimited on one side by cell *f*, and on other side by cells *ee*. At a deeper focus these three cells are free from bacteria, except for a few lying between cell-walls. Similar collections of bacteria along cell-walls may be seen in extreme upper part of picture. Contents of epidermis cells omitted. Slide 412 A9, upper row, sixth section from left. Paraffin embedded section, stained with carbol fuchsin. Drawn with a Zeiss 3 mm. 1.40 n. a. oil immersion objective, No. 12 compensation ocular and Abbe camera.



The time between inoculation and visible disease may be as short as 24 to 48 hours, or as long as 3 or 4 weeks. It varies not only with different organisms, but with the same organism under different conditions. In some species long cultivation on artificial media destroys or greatly weakens the ability of the organism to attack tissues. In other cases a similar reduction of virulence occurs within the host. Experimenting with juicy susceptible plants and such organisms as *Bacillus carotovorus*, *B. oleraceae*, *B. aroidaeae*, *B. melonis*, or *B. hyacinthi* (Heinz), the result of a single needle-prick is often visible in 24 hours, and by the end of the third day the necrosis of tissue is often quite extensive. With the same organisms and in the same host-plants, but in rather woody or somewhat dry spongy tissues, the progress of the disease is slow, and after a slight development it may stop altogether. Potter states that his *Ps. destructans* inoculated into turnips caused very distinct signs of the disease in 24 hours.

For their rapid development most of the soft-rot organisms require tissues full of water. With *Bacillus phytophthorus*, using virulent cultures, susceptible varieties of potatoes, and optimum temperatures, and inoculating by needle-pricks, rot is always visible in 24 to 48 hours, and the entire tuber may be rotted in a week's time, even in dry air. In pear-blight the blackening of the shoots usually occurs in from 3 to 10 days after inoculation by needle-punctures from fresh agar cultures (vol. I, plate 28), but may sometimes be delayed 23 days (Arthur). Much depends on the weather and on the immaturity of the shoots. The pear-blight develops soonest in moist, warm weather and in rapidly growing shoots. In blossom-infections there is a distinct browning in the nectaries in 48 hours, and on the third or fourth day the whole flower collapses and is blackened, together with its pedicel, which has become infected. In the writer's experiments with *Bacterium solanacearum* in 1895 and 1896, blight appeared in young shoots of the potato and tomato in about 4 to 6 days when they were inoculated by needle-pricks from young cultures. On the contrary, in an old and woody tomato plant wilt did not become general until 8 or 9 weeks after the punctures, but then the organism was found to have multiplied enormously in the vascular system, extending to a distance of several feet from the pricked part of the stem. In large tomato plants in a field in South Carolina, during wet weather in July 1895, direct infections by needle-stab induced plain signs of the disease only after many days. Similar tardy results were obtained in Washington in a hothouse in 1909. When Colorado potato beetles were used as carriers of the organism the first signs of the disease in potato appeared in 7 to 9 days from the time the plants were bitten. In more recent experiments with this organism, especially some tests made in 1904 with an extremely virulent strain obtained from a blighting potato, wilt appeared in young tomatoes in 48 hours after inoculation by needle-pricks, and the entire plant was destroyed in 6 days (see vol. I, plate 26). These plants were in pots in the hothouse, were 1 to 1.5 feet high, and were growing rapidly when the stems were pricked.

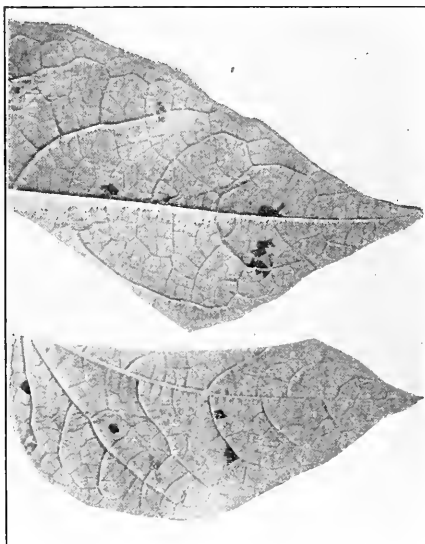


Fig. 18.\*

\*Fig. 18.—Angular leaf-spot on Rivers cotton. Inoculated by spraying Jan. 21-23, 1905. Photographed March 15. Natural size. Spots in water-soaked stage.

With *Bacillus tracheiphilus*, inoculating from young agar-cultures, potato-cultures, or bouillon-cultures into the leaf-blades of cucumbers and other very susceptible plants by means of needle-pricks, the writer has seldom been able to obtain signs of the disease in less than 3 or 4 days. Usually the first signs (wilt and change of color in the vicinity of the punctures) were visible in 5 to 7 days. Occasionally the wilt did not appear until after the tenth day, once it was not manifest until after 21 days, and once not till after 30 days.

In leaves of hyacinths inoculated by needle-pricks with *Bacterium hyacinthi* signs of the disease appeared in from 1 to 3 weeks.

In sweet corn infected in the seedling stage by *Bacterium stewarti*, a period of 1, 2, or 3 months may intervene between the first signs of disturbance in the seedling leaves and the general sickening of the plant, during which, of course, the plant has grown to many hundred times its original weight. There may be an equally long period between local infection and constitutional disturbance in case of sugar-cane attacked by *Bacterium vascularum*.

In Savastano's experiments with the olive-tubercle, knots began to appear upon the young shoots in a little over a month after puncture and were well developed in 2 months. In my own and Mr. Rorer's experiments, incipient knots were frequently visible as early as the end of the second week, *i. e.*, sufficiently developed to be distinguished from the control punctures, and were very distinct in a month, but larger, of course, after several months (see vol. I, plate 2). Once in a later experiment, starting with cultures very recently plated from a knot and introducing the organism by needle-pricks from agar, I observed the beginnings of tumefaction on 5 shoots as early as the ninth day.

In case of the soft-galls, due to *Bacterium tumefaciens*, the writer has sometimes obtained the distinct beginnings of them as early as the third or fourth day, using pure cultures, needle-punctures, and very susceptible tissues such as young shoots of the Paris daisy. Earlier than this it is not possible to decide whether the slight swellings are to result in tumors or are simply a reaction of the plant to the needle-thrust. Ordinarily if the organism is virulent, the tumors are distinctly visible in 8 or 10 days if the tissues are young and tender, but they continue to grow for several months, or even many months.

The bacterial leaf-spot diseases are usually visible in 1 to 2 weeks from the date of inoculation.

#### DURATION OF DISEASE.

Plants show very different degrees of resistance to a bacterial organism once ensconced in the tissues. The soft-rot organisms, as already noted, are usually prompt in their action, and a week or two is often sufficient to destroy the susceptible parts. The writer has seen a good-sized potato-tuber half rotted in 5 days at ordinary autumn temperatures when inoculated with *Bacillus phytophthorus* by means of a few needle-pricks, this too, in a rather dry air; others wholly rotted in 8 or 10 days. Under favorable circumstances, inoculating from a young agar-culture, the flesh of a melon one decimeter in diameter may be rotted wholly by *Bacillus melonis* in 4 or 5 days (Giddings, Smith). In case of the leaf-spots, progress is much slower, and the disease is generally restricted to small areas, *e. g.*, bacterial leaf-spot of the peach, which dry out and fall away from the sound tissue, especially if excised by a cork layer. After plain signs appear, a week or two is sufficient in most cases to accomplish the destruction of the affected part. In cucumbers and muskmelons attacked

by *Bacillus tracheiphilus*, the progress of the disease is rapid after the incubation period has passed. In squashes, on the contrary, the resistance is much greater, and it may be several weeks after a vine shows the wilt before it entirely succumbs. At night, or in moist weather, it becomes turgid, to again collapse with the reappearance of sunlight and dry air. In the growing season, pear-blight is usually a rapid disease, but in the cool weather of autumn and winter there is frequently an almost balanced activity between host and parasite, resulting in what is known as "hold-over" blight. In this way the disease is carried from one growing season to the next. Vascular diseases, such as those of sweet corn and sugarcane, already mentioned, kill the plant very gradually, if it is of good size when infected or when the constitutional signs first appear, but after the vascular occlusions have reached a certain volume the destruction of the plant is speedy. In maize, which has reached this stage, the leaves dry out within a few days, and the green stem then shrivels. In case of the olive-tubercle, the tree as a whole does not, so far as we know, become infected but only particular parts of it, yet there may be wide metastasis especially in young trees. Individual knots live for several months, and frequently portions of them for several years, the knot enlarging from some particular part which has not been injured beyond the power of cell-division. Terminal twigs girdled by tubercles are frequently starved and die, but not very promptly. Knots and cankers due to bacteria are generally of slow progress and correspondingly long duration. The life of a shoot of chrysanthemum, sapped by a big tumor due to *Bact. tumefaciens*, varies from 6 months to a year or more. Often the plants live many months. Peach trees attacked by crown-gall generally live for several years. Galled apple trees may live indefinitely. In the recently discovered tuberculosis of the sugar beet due to *Bacterium beticola* (Vide Crown Gall, etc., Bull. 213) decay is rather prompt.

#### FINAL OUTCOME.

Plants, like animals, are affected to very different degrees by the various bacterial parasites. This must be apparent from what has been said under duration of the disease. In the animal world there are protracted bacterial diseases and rapid ones, diseases terminating fatally or ending in recovery. The same is true of plants. The simplest cases, perhaps, are the stomatal infections resulting in leaf-spots and fruit-spots. The leaves are more or less disfigured, and the fruit may be destroyed or so spotted as to be unsalable, but generally it is beyond the power of the organism to destroy the plant, or even to render it wholly unfruitful. In bacterial blights, such as that of the mulberry or pear, much larger portions of the plant may be destroyed, twigs or even large branches, and yet it may recover. In a majority of cases, after running a



Fig. 19.\*

\*Fig. 19.—Coconut budrot of Eastern Cuba. Outer enveloping leaf sheaths removed to show condition of inner undeveloped leaves—sound below, rotted above. Tree No. 11. Bud itself not dead, but enveloping sheaths rotted. Color of decayed part was a mixed gray and brown. Photographed by the writer at Baracoa, Cuba, April 20, 1904. One-third natural size.

certain course, which is usually not shorter than several weeks, the disease stops and the organisms which caused it are then found to be dead in the blighted tissues (pear-blight). This, however, does not seem to protect the tree from new infections the following year, *i. e.*, the disease is not self-limited and protective from new infections like the eruptive fevers. Not infrequently rapidly growing, juicy trees of pear, apple, quince, loquat, and mulberry are killed outright in the course of one season if left untreated. Even whole orchards have been destroyed, as in Georgia and California. Olive-tubercle also sometimes kills young trees, but more often it kills only some of the smaller branches and renders the tree unfruitful. Certain infections seem to kill almost infallibly. This is true of *Bacillus tracheiphilus* in musk-melons and cucumbers, and of virulent strains of *Bact. solanacearum* in young



Fig. 20.\*

tomatoes, potatoes, and egg-plants. Whole fields of potatoes, tomatoes, and tobacco when young may succumb quickly to this disease, particularly in moist soils containing root nematodes. In carefully made inoculations on young plants, using either of these organisms, at least 95 per cent of the infections are promptly fatal, *i. e.*, within 2 or 3 weeks from the first visible signs of the disease, and sometimes much sooner (see vol. I, plates 24 to 27). Old plants are more resistant, especially to *Bacterium solanacearum*. In the same way old and slow-growing cabbages are rather resistant to *Bacterium campestre* and may not be wholly destroyed, but young and rapidly growing plants are very apt to die either from the direct effects of the parasite or from the action of the soft-rots which follow it.

\*FIG. 20.—Coconut budrot of Eastern Cuba. Outer leaf-sheaths removed to show inability of diseased terminal bud to support its own weight. Tree No. 10. Photographed at Baracoa, Cuba, April 18, 1904. Natural size. Photographed in a room at 2 p. m., raining, with Cramer's instantaneous plate, Zeiss double protar lens, series VIIA, stop 256, time 30 minutes.

## TISSUES ATTACKED.

In what we may consider as the lowest type of these diseases the parenchyma-cells of storage tissues are the parts principally attacked. Often these are aggregated in fleshy organs which have reached maturity and ceased to grow but abound in water, amid, proteid and carbohydrate substances, designed for the green plant which is to be developed the coming season. Buds, bulbs, tubers, rhizomes, and various swollen underground parts of mixed structure are good examples. Examples of such diseases are certain soft-rots of potato-tubers, Jones's carrot-rot, Metcalf's rot of sugar beet, and the coconut bud-rot (plate 5, and figs. 19 and 20). Usually they do not appear in green, growing parts. They destroy the tissues by softening the middle lamellæ. In a little higher grade of essentially the same type of disease, the green parts of plants are also attacked, *e. g.*, iris-rhizome-rot, Appel's potato-rot, lettuce-rot, calla-lily-rot. All these organisms require tissues rich in water, otherwise they refuse to grow or make very little headway.

A grade higher in the scale, perhaps, are those bacteria which attack the parenchyma of stems, roots, bark, green leaves, etc., but can do so only when the tissues are in a rapidly growing, actively dividing condition. They may retain a foothold for some time thereafter, in exceptional cases, but their power for evil is limited to a short period of the growing season. Black-spot of the plum and pear-blight (vol. I, plates 28, 29) are good examples. All of the leaf-spots are primarily diseases of the parenchyma, and some of them are limited to quite restricted areas of the parenchyma, *e. g.*, leaf-spot of the carnation, larkspur, soy-bean. Other diseases like pear-blight and Aderhold's cherry-blight often extend a long distance through parenchymatic tissues. In case of the pear the upward or downward movement of the bacteria in the bark may be several meters, and the sidewise movement is often sufficient to girdle and kill large limbs or even the whole tree.

In general, however, destruction is more extensive when the organism is able to attack the vascular system as well as the parenchyma. Then we have phenomena of occlusion, and marked interference with transportation of water. There are intermediate forms and transitions of various sorts as might be expected. The pear-blight organism, so far as I know, seldom follows the vessels, expending its energy rather on the cortical parenchyma. Some leaf-spot bacteria seem to use the vessels more than others.

Appel's potato-rot organism (*B. phytophthorus*) makes some use of the vessels of the stem, but seems more at home in the parenchyma. On the contrary the organism causing Stewart's sweet-corn disease, to take a very striking example, develops principally in the vascular system and destroys the plant from this vantage ground. This is true also of *Bacillus tracheiphilus* and *Bacterium vascularum*. The same is true of *Bact. solanacearum*, in potato and tomato, only here the organism finally floods out into the tissues of pith and bark much more than in the other cases cited (fig. 1). *Bact. solanacearum* and *Bacillus phytophthorus* may be compared and contrasted in this particular since both attack the potato. Both make use of the vessels, but the former does so much more extensively and destructively than the latter; the one is primarily a vascular disease, the other a parenchyma disease; one destroys the stem by occluding the vessels, the other by rotting it off at the surface of the earth. All vascular diseases make pockets in the parenchyma, but in most cases these are only in close proximity to the vessels (fig. 6) and after the latter have been occluded and destroyed. In very soft tissues such as those of watery, fast-growing tomato shoots, *Bact. solanacearum* finally makes very extensive closed cavities, often honey-combing both pith and bark for many centimeters. *Bacterium vascularum*, though restricted pretty closely to the bundles for long distances in the maturer parts of the stem of sugarcane, often excavates extensive closed cavities in the very soft undeveloped parenchyma under the terminal bud. The gum diseases rupture the bark and ooze extensively on the surface. Pear-blight does this also to a lesser degree. Some, perhaps all, of this class of bacteria reach the surface through fissures due to surface tensions set up in dead tissues by

the parts still alive and growing. Others reach the surface of the living plant, if at all, principally through natural openings, *i. e.*, stomata or lenticels.

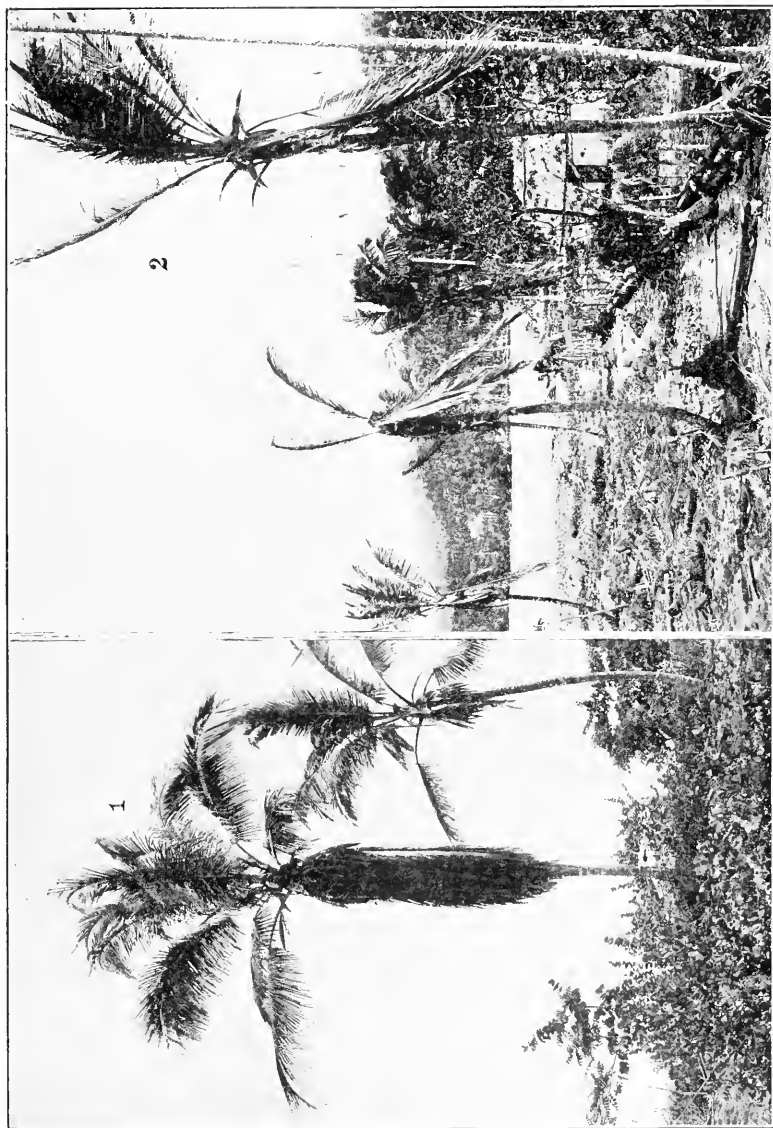
I do not know of any bacteria confined to the sieve-tubes, or particularly at home therein, but probably this only amounts to saying that the whole field has not been surveyed.

Parasitic bacteria, limited to the woody tissues of trees and shrubs or supposed to be peculiarly at home therein, have been described but are unknown to the writer. Examples cited in literature are *Mal nero* of the vine, and Janse's disease of dadap trees, both abundantly doubtful etiologically. The writer has seen bacteria-like bodies in fossil woods, but these woods may have been occupied by them after submersion in some swamp. He has also seen a yellow Schizomycete very abundant in the wood of pear trees attacked by pear-blight, but this had no pathogenic properties. Viala and Ravaz found a very abundant multiplication of a Schizomycete in the vessels of vine cuttings buried in sand to be used later as grafts, but the organism was unable to propagate itself in the living, growing plant when these cuttings were used either as grafts or scions, and cultures made from these bacteria had no pathogenic power when inoculated into the vine. There is no apparent reason, however, why the wood of living trees should be wholly exempt from the attacks of bacteria, and cases will probably be discovered in which bacteria are confined pretty closely to the woody tissues. They must of course be sorts able to live on a minimum quantity of water.

The highest type of bacterial disease, and the most interesting from many standpoints, is that in which all the growing tissues, pith, wood, cambium, and bark are involved, and are stimulated into abnormal, excessive growth, death occurring only after extensive hyperplasia. These overgrowths may attack roots or shoots. Good examples are olive-tubercle, pine-tubercle, beet-tubercle, the daisy knot, and crown-gall of the peach. In the olive, oleander and daisy they often occur on the leaves. Metastatic tubercles and secondary tumors occur. This type will be discussed more at length under Reactions of the Plant. There appear to be two forms of these overgrowths. In the olive tubercle, bacterial cavities occur and the organism is abundant in them, and is easily observed wedging its way between cells. In the crown-gall no such cavities have been observed, the causal organism is difficult to detect with the microscope, and its location in the tumor tissue appears to be unlike that of the olive-tubercle organism. Moreover, plate cultures show that it is not very abundant in the tissues, at least in a viable form. The olive tubercle organism occupies intercellular spaces. The crown-gall organism occurs within the rapidly dividing cells, as in case of the root-nodule organism of Legumes, but less abundantly and does not form a bacterial strand.

#### MASS-ACTION OF BACTERIA.

A few words are necessary on the mass-action of bacteria. It is a common observation, one made by the writer at least a hundred times, that in culture-media not exactly adapted to the needs of an organism, a scanty inoculation may not give any growth—not even after a long time—whereas a copious one will lead to a growth which gradually clouds the fluid or covers the solid. The penetration of bacterial strands from cell to cell in the root-nodes of Leguminosae is another example (figs. 21, 22). The only explanation I can think of is that a multitude of the bacteria are stronger than a few, and thus by union are able to overcome obstacles too great for the few. The same fact comes repeatedly to the attention of the animal pathologist as a result of his inoculations. The animal body, we must assume, is often able to overcome and destroy a few hostile organisms, where it would not be able to defend itself against many; otherwise whole races would be exterminated by natural infections. The same is undoubtedly true in plants. The *modus operandi* in plants is not altogether clear. We may advance several hypotheses: (1) The formation of a resistant cork-layer before the bacteria have multiplied to such an extent as to prevent



Coconut palms in middle and late stages of bacterial budrot. Photographed by the writer in eastern Cuba in Apr. 1944.  
 (1) From a small grove on low land at mouth of Rio Miel near Baracoa. Relaxed position of lower fronds, falling of nuts green, and wilting of undeveloped young leaves are typical signs.  
 (2) From south side of bay at Mata. Trees dead and dying.





cell-division; (2) the destructive action of antiseptic plant-substances, *c. g.*, acids, before these can be neutralized or otherwise destroyed by the substances produced by the multiplying bacteria.

In some instances, the introduction of a very considerable mass of bacteria seems to be necessary to induce disease; in other cases a very few are sufficient. It would be extremely interesting to know the minimum number capable in any given case of inducing disease. This could be determined easily by the dilution method, and still more readily and with

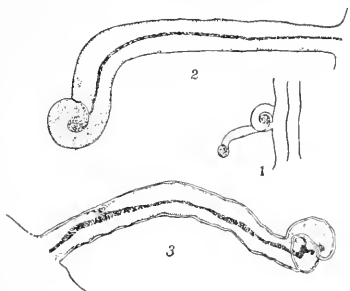


Fig. 21.\*

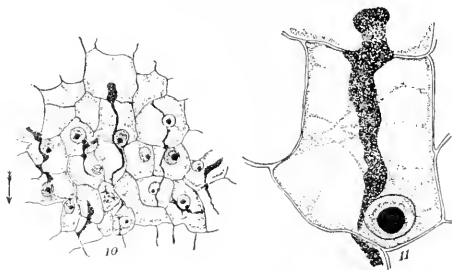


Fig. 22.†

absolute accuracy by the use of Barber's apparatus, but no one seems to have made any exact experiments. Good organisms for experimental purposes would be *Bacillus phytophthorus*, *Bacillus tracheiphilus*, *Bacillus amylovorus*, and *Bacterium campestre*, care being taken, of course, to select sensitive varieties and susceptible tissues, and to have all other factors comparable.

#### SECONDARY TUMORS AND METASTASIS.

Secondary foci of overgrowth occur in the olive and in the daisy as the result of internal infection. The writer has obtained them frequently in both plants by pure culture inoculations (plates 6 and 7, and fig. 23). The organisms pass through the tissues of the stems or leaves and set up irritations which lead to hyperplasias in particular spots in the deeper tissues. These tissue enlargements, later on, break through to the surface. Sometimes these secondary growths arise at a considerable distance from the primary tubercle. In case of olives inoculated in 1910 the writer observed numerous deep tubercles develop at a distance of 1, 2, and 3 feet from the point of inoculation within a period of 7 months in actively growing plants, both down and up the shoot. The movement is more apt to be up the stem or leaf, *i. e.*, with the transpiration current, than down the stem.

In the olive a distinct channel of infection is traceable from the primary to the secondary tubercle. This is usually (so far as observed) a narrow pathway in some part of the inner wood, the tissues being more or less stained and disorganized, and the bacteria present in abundance and easily demonstrable without staining. Whether similar downward

\*FIG. 21.—Three figures from Peirce's paper:

- (1) Two root-hairs of Bur clover infected by nodule bacteria, showing characteristic bending at point of infection.  $\times 50$ .
- (2) The lower of two root-hairs in 1, showing mass of bacteria in concavity of coil and infection thread running from this point through the hair.  $\times 300$ .
- (3) Another infected and coiled root-hair, infection thread growing close to nucleus of hair.  $\times 300$ .

†FIG. 22.—Two figures from Peirce's paper on Root Tubercles of Bur Clover:

- (10) Section of a tubercle near meristem. Direction in which meristem lies is indicated by arrow. Section stained by Fleming's triple stain and differentiated, after anilin gentian violet, by Gram's iodine. Course of infection threads is definitely toward tubercle-meristem and generally toward nucleus of cell entered.  $\times 200$ .
- (11) One cell from 10, showing solid infection strand (zoogloecae) in which separate bacteria can be distinguished.  $\times 1000$ .

channels occur in the bark has not been determined. The channels in the wood, which probably begin with an occlusion of some of the spiral vessels, generally occupy but a very small portion of the stem, although they are easily visible on cross-section even to the naked eye as small brown specks and on longitudinal section as a dark line bordering the pith and connecting the two tumors.

In the Paris daisy attacked by *Bacterium tumefaciens* I have been able to obtain leaf-tumors in a considerable portion of my inoculations by making a single needle-prick into the soft young stem below the leaf, the infected needle being thrust into one of the three leaf-traces. A primary tumor results at the point of inoculation on the stem, and some weeks later a secondary one develops in the inner tissues and subsequently bursts through the upper surface of the leaf, *i. e.*, through the petiole or midrib. Sometimes there are a series

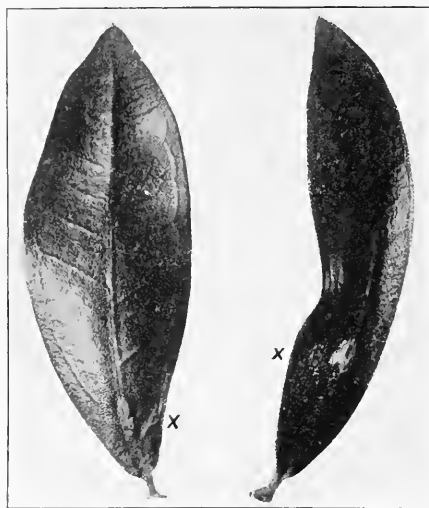


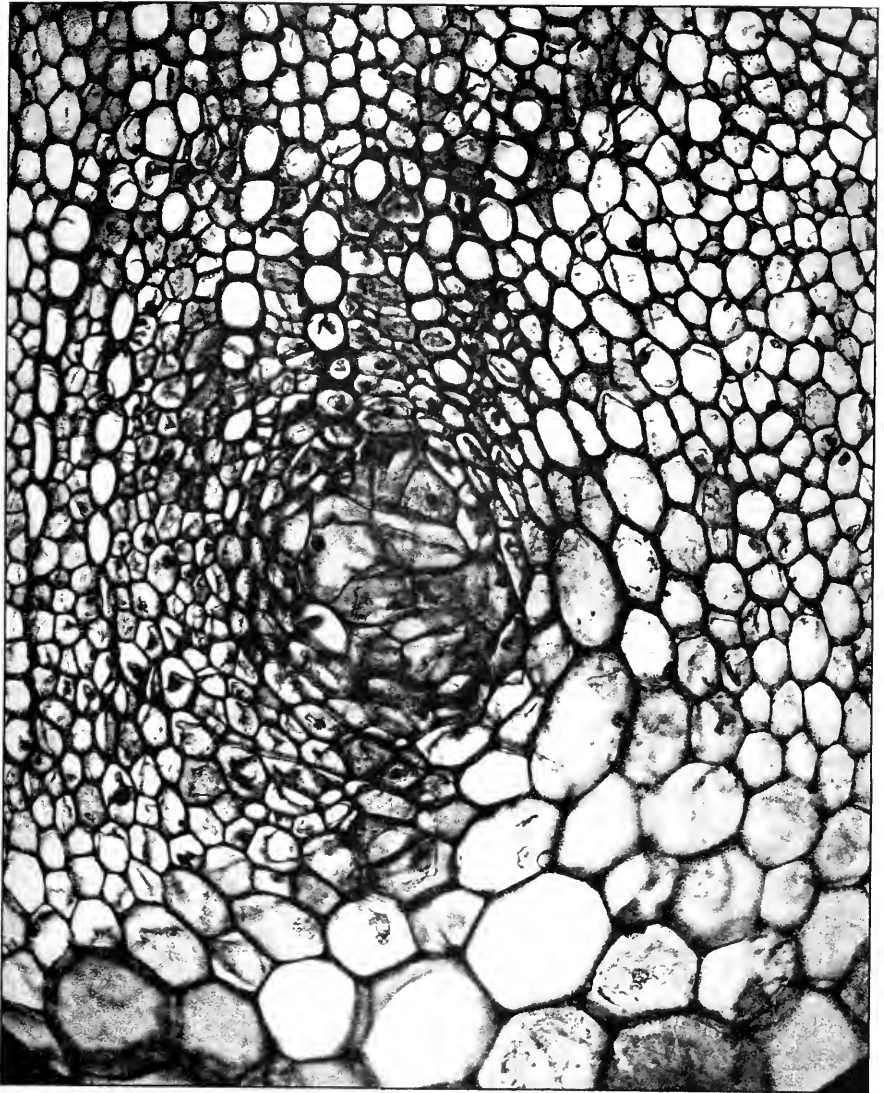
Fig. 23 \*

of such internal secondary tumors (fig. 24). In young daisy plants inoculated January 13, 1911, upon the stems, secondary (internal) tumors appeared upon the leaves very promptly and after 16 days were visible 7 to 10 cm. away from the primary tumor. The movement in this case was upward and the little tumors had not yet burst through the midrib. Sections were cut and the internal tumor tissues studied in these midribs, and motile bacteria-like rods in small numbers were seen inside the cells in the gall tissue.

The channel of infection in this case is not so easily traced. There is no plain disorganization of tissues, and no brown stain such as occurs in the olive, and although I have studied a good many sections I do not yet know through what tissues the channel of infection passes or whether there is any definite channel. It is more likely that the tumor tissue itself carries along the organism within the rapidly dividing cells, there being a chain of tumor cells all the way from the primary to the secondary tumor.

Since the above paragraph was written additional observations have been made confirming this hypothesis. A strand of tumor tissue has been discovered connecting the primary tumor with the secondary tumors. This is usually in the inner wood or at the junction of pith and wood. Moreover, when the primary tumor is in the stem and the secondary tumor is in the leaf, the latter tumor does not have the dorsi-ventral structure of the leaf in which it is growing but rather the structure of an imperfect stem. In cross-section it is circular or nearly so, and is composed of a pseudopith of tumor tissue beyond which is xylem surrounded by cambium and what I take to be phloem. Often remnants of the petiole or midrib adhere to the surface of the tumor as a shell or as wing margins, in which one finds the unchanged dorsi-ventral structure of the leaf. The strand or wedge of tumor tissue proceeding from the primary tumor grows through the inner tissues of the stem, petiole, and leaf-ribs much like a foreign body, developing the secondary tumors from its substance in places of least resistance, or where the food supply is most abundant. These

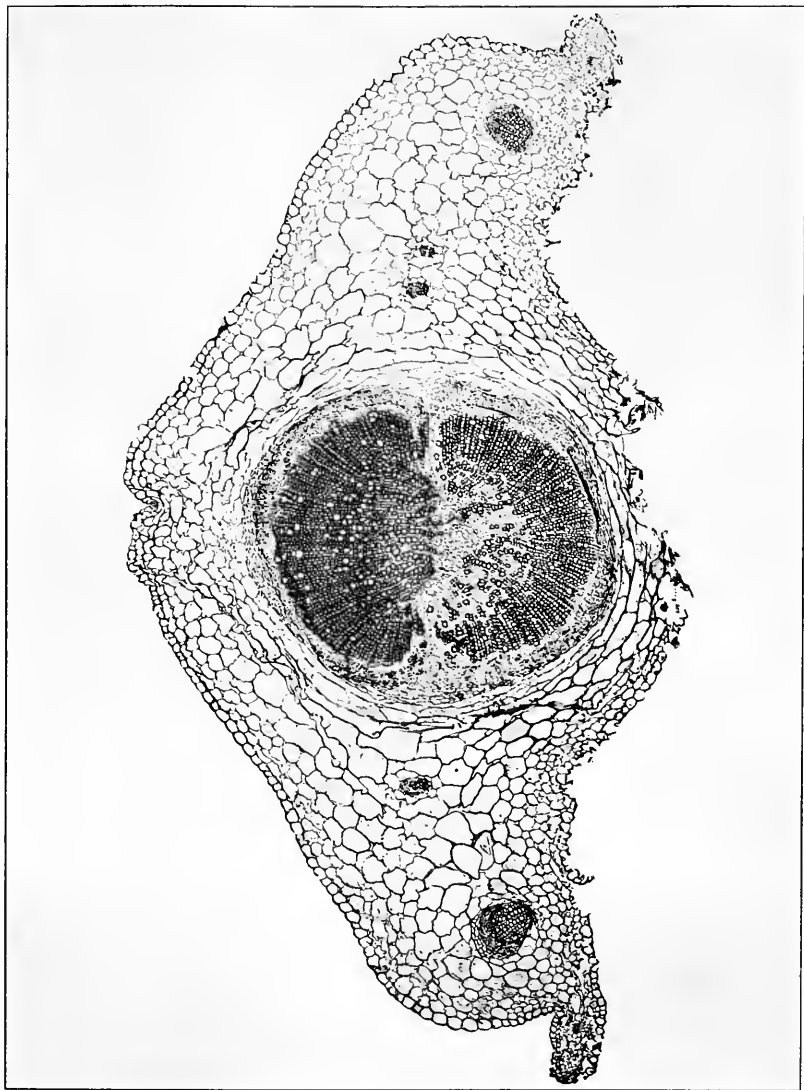
\*Fig. 23.—Metastatic small tubercles (nearly erumpent) on midrib of olive leaves at xx. Inoculations were made Nov. 26, 1907, on stems about 0.5 inch below nodes, and growths developed at these points: Subsequently, these leaf-tubercles developed from within. Inoculated plants Nos. 424a and 412. Photographed Feb. 29, 1908.



Crown Gall of Daisy.

Cross-section of stem of an inoculated plant between a primary tumor and secondary tumors, showing a tumor strand in the inner wood in the center of the figure. Pith below. The secondary tumors are outgrowths from such strands. See next plate and page 72. Slide 639 A 6.

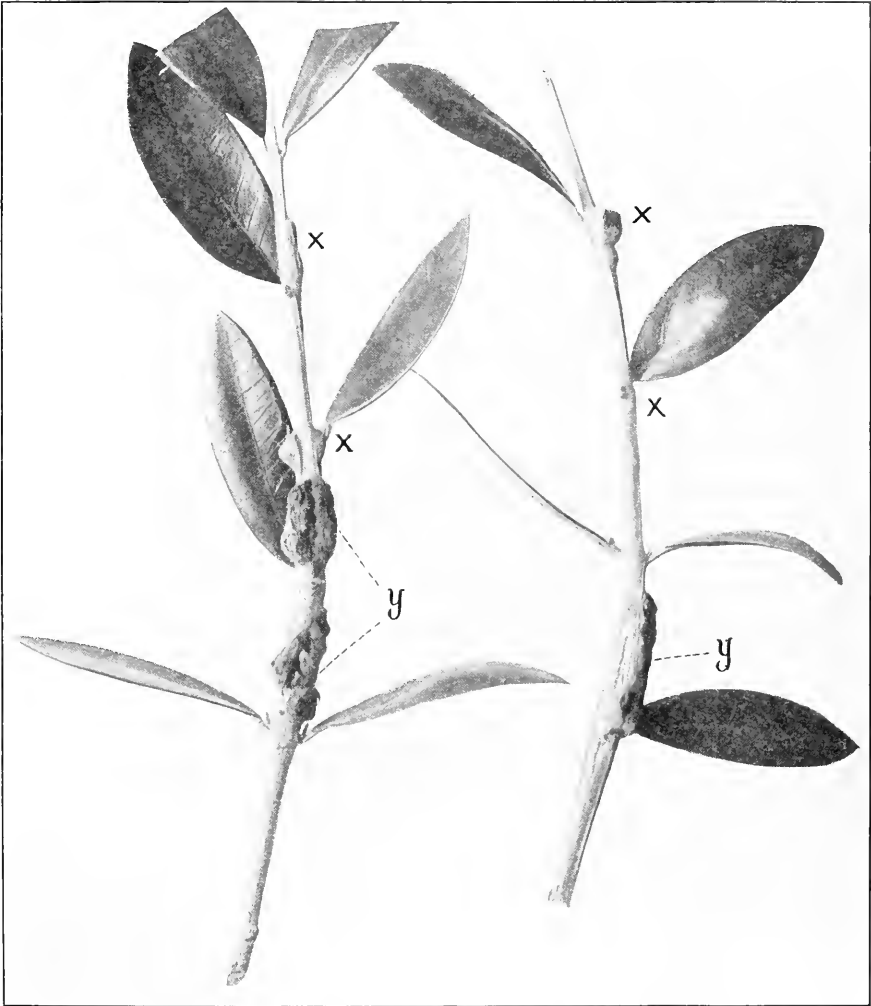




Crown Gall of Daisy.

Cross-section of petiole from an inoculated plant showing the central leaf-trace enlarged and converted into a pseudo stem by the parasitism of *Bact. tumefaciens*. The reason for the stem structure of the leaf tumor lies in the fact that the tumor-strand infecting this leaf-trace is an outgrowth from a primary tumor on the stem. The remainder of the petiole is normal. Slide 634 B 10





Tuberculosis of Olive.

Shoots of olive inoculated Nov. 26, 1907, at yy by needle-pricks. A pure culture of *Bact. savastanoi*, 3 days old, was used. Photographed Mar. 13, 1908. Metastatic tumors in process of development at xx. Channel of infection in vessels next to pith.







Crown Gall on Daisy.

Two tumors on the stem of a Parris daisy as the result of an inoculation of *Bact. tumefaciens* by needle-pricks, and on a branch above the upper one a secondary tumor on the petiole of a leaf. Age of primary tumors about three months; that on the leaf is much younger, perhaps four weeks old.



internally developed secondary tumors contain the parasitic bacterium the same as the primary tumors, but it is not abundant in any of the tissues. The bacterium causing the disease has been found also sparingly in the strand connecting primary and secondary tumors.

Much difficulty has been experienced in staining *Bact. tumefaciens* in the tissues of the daisy, and ideal preparations are yet to be obtained. It would seem from an examination of numerous slides, and also from the results of many poured plates, that the organism occurs in the tissues of the tumor in rather small numbers, especially as compared with the organism causing the olive-tubercle. From what I have seen I believe it occurs only inside the parenchyma cells, stimulating them to divide and passing on from mother cells to daughter cells in this manner.

The development of these tumors depends on a very delicate series of adjustments between the attacking organism and the host cells. My present hypothesis as to the mechanism of the tumor development in crown-gall is as follows: Through wounds which have not injured the cells beyond the power of recovery (needle-pricks in case of my experiments) the bacterium gains an entrance into the cell; here it multiplies rapidly for a short time; its further growth is checked by the appearance of acid to which it is very sensitive—this acid being developed in the cell as a by-product of the bacterial action on sugar; the first effect of the acid is to inhibit the further growth of the bacteria and consequently there never can be very many bacteria in any individual cell; the continued action of this acid on the bacteria leads to the production of involution forms (clubs and Y's) and finally a portion of these bacteria are killed outright, but the concentration of the acid is not sufficient to destroy the host cell; the nucleus of the latter now divides, either under the direct stimulus of the acid, or under the influence of bacterial endotoxins which now for the first time have been liberated, *i. e.*, have come into contact with the nucleus by diffusion through the permeable membranes of the dead bacteria; during the cell-division the bacteria are carried over into the daughter cells, where under the new conditions those not destroyed by the acid multiply rapidly for a short time; then in turn their growth is checked by more acid, whereupon ensue the other changes ending in another cell division. In this way is developed first the primary tumor, then the strands penetrating the sound tissues in various directions, and finally the secondary tumors, which I have elsewhere called metastatic tumors, but which are probably not so in the sense that loose tumor cells migrate from the primary tumor to

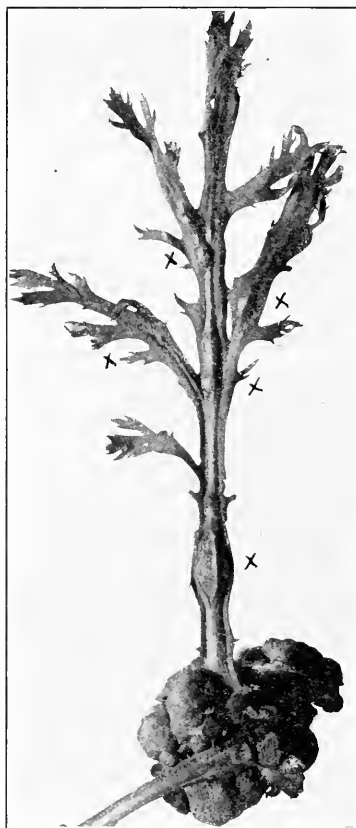


Fig. 24.\*

\*FIG. 24.—Leaf of Paris daisy (*Chrysanthemum frutescens*) developing internal tumors at points marked by letter x and beyond. The lower one has split open surface of rib, others are still sub-epidermal. Source of infection was the stem-tumor here shown, which was induced by needle-punctures introducing *Bacterium tumefaciens*. Photographed Feb. 12, 1908.

form them, since the circulation in plants is not as well adapted as in animals to this sort of migration. The stages outlined above take place with great rapidity since in very susceptible tissues, *e. g.*, young, rapidly growing sugar-beets, it is possible by means of a few needle-pricks to obtain a tumor as large as a man's fist in 5 or 6 weeks.

These phenomena represent to me an entirely new type of bacterial disease. They seem to me also to throw a flood of light on the mechanism of the development of malignant animal tumors, making it likely that they also are due to parasites having similar relations to the cells of man and the lower animals.

The facts underlying this hypothesis may be summarized as follows:

(1) The crown-gall disease is of bacterial origin beyond reasonable dispute, as shown by hundreds of poured plates and pure culture inoculations. It is also a neoplasm rather than a granulomata (vide evidence advanced in Bulletin 213).

(2) The bacteria can not be found readily in the tissues by means of microscopic examinations although the poured-plate method shows that they occur there, and the vessels and intercellular spaces being free from any granules whatsoever, the bacteria must occur inside of the cells, forming some portion of the cell-inclusions.

(3) The poured plates confirm the microscopic examinations. They show that the bacteria are not abundant in the tissues. They also show that these bacteria often occur in the tissues of the tumor in a moribund state, requiring 4 to 6 days or more to recover and develop colonies on the agar, although when once recovered they grow in second and subsequent transfers as promptly as other organisms.

(4) In flasks containing water, peptone, grape-sugar, and calcium carbonate the organism (from the daisy) produces an abundance of acetic acid.

(5) Chemical analysis shows an excess of acid in the tumor tissue as compared with sound parts of the same plants (daisy, sugar-beet), but up to this date a sufficient quantity of the tumor for a definitive quantitative test (10 kilos or more) has not been available. If acetic acid is formed in the tumor cells, it must be in minute quantities, and it might be oxidized by some subsequent action of the host protoplasm so as not to be recoverable on chemical analysis.

(6) In artificial cultures club-shaped, Y-shaped, and variously branched bodies can be produced at will by adding small quantities of acetic acid.

(7) Similar forms occur in the tissues of the tumor, and while I have not seen them in the cells they can be obtained on sterile slides in small numbers by making sections of tumors and allowing them to diffuse in sterile water for a few minutes.

(8) When too strong a dose of acetic acid has been added to the agar, or bouillon cultures, the Y's and other involution forms can not be resuscitated by means of agar poured plates, but when the dose has been properly adjusted a portion of the bacteria may be recovered in poured plates, the colonies coming up slowly the same as when material is taken from the interior of the tumors.

(9) Finally, the statements respecting the tumor strand, the anatomy of the secondary tumors, and the occurrence of the bacteria in these latter are supported by many observations and experiments.

Schiff-Giorgini first clearly recognized metastasis in the olive, although earlier Savastano pointed out that some tubercles develop superficially and others from the deep tissues.

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SOLVENT ACTION OF BACTERIA—DESTRUCTION OF MIDDLE LAMELLÆ—BACTERIAL  
SOLUTION OF CELL-WALLS—FERMENTATION OF CELLULOSE—  
DESTRUCTION OF WOOD.

Next to crushing and splitting, due to the rapid multiplication of the bacteria in closed spaces, solution of the middle lamellæ uniting cell-walls is probably the most widespread and simple action of bacteria on plant tissues. This is common in a great number of diseases, but it is not always possible clearly to separate lysis from tension-splitting, when the bacteria are multiplying rapidly in a given tissue and must have room. An excellent example of the separation of cells by a solvent action on the pectic matters composing the middle layers of the common wall may be seen in various rots of potato-tubers. A few days after an inoculation the tissue softens, and if it is then washed in water the cells float free, their starch content remaining unacted on (fig. 25). Potter asserts this solution to be due, in case

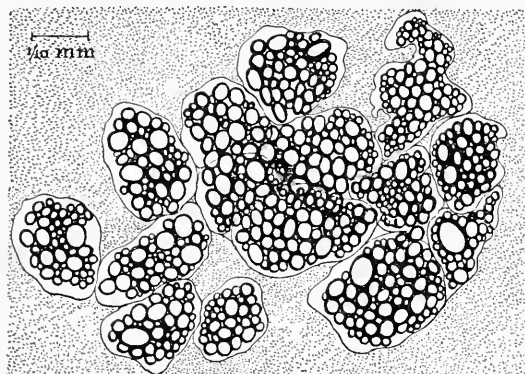


Fig. 25 \*

of a turnip rot which he studied, to the presence of oxalic acid. The writer found oxalic acid had no solvent action on slices of turnip, but that ammonium oxalate softened the middle lamellæ decidedly. Inasmuch as a part at least of any oxalic acid liberated by any Schizomycete of this type would be converted into ammonium oxalate by the evolution of ammonia due to the continued growth of the organism, it is not unlikely that ammonium oxalate is the substance, which in some cases, dissolves the middle lamellæ.

It would seem, however, that in many cases a specific enzyme, a

pectase, must be the solvent body. Vide paper by Spieckermann and papers by Jones. Once or twice in earlier papers the writer has used the word "cellulose" loosely, in the old way, for cell-wall, of which it forms, however, only a part. When the middle layers of pectic origin have been destroyed there yet remains a wall of cellulose surrounding each cell. Is this permeable to bacteria? Can any of the bacterial plant parasites dissolve it? No such crucial studies have been given to the subject as Omélianski, for instance, has given to the solvent action of the anaerobic organisms of marshes known as the methane bacteria. It has been demonstrated that the marsh-gas bacteria destroy cellulose in large quantities, but nothing like that, of course, occurs in the diseases in question. All we know definitely can be expressed in few words.

Bacteria certainly find their way into the interior of cells which have not been crushed or mutilated. The evidence of this is the fact that they are so found in great numbers and inside cells under conditions which seem to preclude entrance through wounds of any sort. How do they enter? Their entrance is an extremely difficult thing to observe owing to their

\*FIG. 25.—Softened tissue from interior of a potato tuber inoculated with *Bacillus phytophthorus* and kept for 6 days at about 25° C. Cells have separated, by solution of middle lamellæ, but starch grains are intact. Oct. 26, 1906.

small size and the great liability to misinterpretation, *i. e.*, in sections there is often an opportunity for differences in judgment as to whether a particular bacterium actually lies in or over a wall; has really passed part way through the wall by a solvent action of its own; or has only been dragged or flooded a little way out of its original position during the preparation of the slide. Potter took the former view and figured a case of a single bacterium halfway through a cell-wall, but I have yet to meet a plant pathologist who has been convinced by his figure. Probably by mass-action the bacteria push or dissolve their way through pits or similar very thin places in the wall, but the demonstration is hedged about with difficulties. Some of them are at the limit of vision, and might perhaps enter through openings too small to be seen, *i. e.*, of a diameter less than a wave-length of light. That they do enter in some way is certain. The most striking example, perhaps, is the voluminous intracellular occupation in the root-nodules of Leguminosae. Here the cells are often crowded with bacteria with no visible opening for entrance. They seem to enter by mass-action, the bacteria being compacted into strands. Often there is a trumpet-like expansion where the strand touches the cell-wall. The writer has seen what appears to him to be a similar dense occupation of unruptured cells in the bark of the pear attacked by *Bacillus amylovorus*. The particular tissue which shows this to best advantage is the pitted collenchyma toward the outer part of the bark. In a few cases it has seemed as if bacteria could actually be traced from one cell into another across a narrow pit, but of the absolute correctness of this view I have not yet fully satisfied myself. Moreover, unless I am mistaken in my interpretation *Bact. tumefaciens* occurs commonly in the closed cells of the rapidly multiplying parenchyma of crown-galls. In crown-gall we do not know, except in case of the initial wound, that there is ever any penetration of cell-walls, rather, as already described, it would seem that the bacteria are carried over from mother cell to daughter cells at the time of cell-division. Other examples of the entrance of bacteria into closed cells are shown in figs. 81 and 120.

In quite a good many diseases large cavities arise in the interior of the plant, the cells being crushed and crowded aside, the walls becoming more and more indistinct until they do not any longer yield the cellulose reaction, and in some cases they seem to have nearly or quite reached the stage of dissolution and actual disappearance, as in black rot of turnips and wilt of cucumbers. Whether they ever quite disappear is a subject for future inquiry. None of the bacteria I have tried have any solvent action on filter paper or cotton fibers, but this, of course, is beside the main issue, since there are several kinds of cellulose, some easier of solution than others.

Nothing is known by the writer respecting the solvent action of bacteria on lignin and cork. Janse asserts that the lignin of *Erythrina* roots is dissolved by bacteria. The writer tried in vain to obtain material of the diseased *Erythrina* roots for study.

The early history of the solvent action of bacteria on cell-walls, so far as it relates to plant pathology rather than to chemistry, is summed up in the writings of Davaine and Van Tieghem.

As early as 1866, Davaine succeeded in rotting certain plants by inoculating them with infusions containing bacteria. These experiments antedate those of Van Tieghem by more than a decade. He called his organisms *Bacterium putredinis*.

In 1879 Van Tieghem detailed the success he had had in rotting land plants with his *Amylobacter*. Aquatics resisted. The meristematic tissue was that most easily attacked. The following are two pertinent paragraphs:

Are the membranes of vegetable cells all attacked by *Amylobacter* indifferently? By no means. I know only one state in which all the cells of all plants are equally dissolved by it, no matter how thick they may be, *viz.*, the embryonic state. As soon as the plant has specialized and solidified its tissues by development, profound differences are noticeable [p. 27].

But it is by no means the same in submerged phanerogamous aquatics. Here the cellulose of all the elements of the stem and of the leaves resists *Amylobacter*, and for these kinds of plants resistance is a necessity of existence [p. 28].

The cellulose of mosses, selaginellas, hepatics, lycopods, and of the fronds of ferns also resists.

VAN Tieghem (1884) observed that when bacterial decay attacked the cut ends of plants immersed in water, it was not limited exactly to the part under water, although it did not make any great progress in the part above water. This led him to make inoculations in various plants, using what he calls the spore-bearing *Bacillus amylobacter*. He inoculated potato tubers by means of punctures and placed them in a thermostat at 35° C. Those which received deep punctures gave the best results. There was bacterial growth, the formation of gas, and finally the decay of the whole interior of the tuber. Similar results were obtained with peas first soaked in water and then punctured so that the cotyledons were injured. The grains of starch remained unaltered, but the cell-walls were broken down. If the bacteria were simply placed under the teguments of the pea without injuring the cotyledons or the embryo there was usually no result. He then tried inoculation of fleshy plants, leaves of Crassulaceae (*Escheveria*, etc.) stems of Cactaceae (*Cereus*, *Opuntia*, etc.), and the fruits of Cucurbitaceae (cucumbers, etc.). The leaves of Crassulaceae and the stems of the Cactaceae exposed in a well-heated room gave no result, although frequently re-inoculated, but rotted rapidly when plunged into oil after inoculation. The fruits of cucumber and melon gave an entirely different result. There was a rapid development of the bacteria and simultaneous destruction of the tissue. In a word, the same result as with potato tubers.

Van Tieghem also tried injecting these bacteria into various submerged aquatic plants (*Vallisneria*, *Helodea*, *Ceratophyllum*) "but always without result. The plant remained sound in all its parts."

The first critical study was by Spieckermann (1902). The following paragraphs on the mechanism of the entrance of bacteria into the plant are condensed from his *Beitrag zur Kenntnis der Bakteriellen Wundfaulniss der Kulturpflanzen* (Landw. Jahrbücher, 31 Bd., pp. 163-174). The organism, which is a white, liquefying, Gram-positive, non-sporiferous 1-flagellate, milk-curdling, acid-forming, nitrate reducing Schizomycete capable of rotting various kinds of vegetables, will be considered in its proper place under soft rots.

The parasitism of the organism depends on its ability to dissolve the middle lamella and to produce a poison which is deadly to the protoplasm. The solution of the middle lamella is brought about by an enzyme which is still present in the expressed juice of decayed plant parts after the death of the bacteria. The dissolving power which this sap still possesses is destroyed by boiling.

For isolating the enzyme the expressed juice of decayed carrots, potatoes, and onions was used. The vegetables to be inoculated were very carefully washed with sterile water, cut in two, and streaked on the cut surface with a large number of bacteria from a 24-hour old agar culture. At the end of 36 hours in a damp chamber the vegetables were completely soft-rotted. The decayed mass was scraped out from the unchanged epidermis with a sterile spatula and mixed with an equal weight of sterile water. The mixture was extraordinarily viscous. The carrot and onion mixture were strained through a towel and then filtered through glass wool. The potato was centrifuged and then filtered. The expressed sap obtained in this way was always very viscous and heavily clouded with bacteria. The reaction to litmus was the same as that of the decayed mass itself, *i. e.*, the potato juice was neutral or alkaline, the onion juice acid, the carrot juice neutral or slightly acid.

In order to do away with the action of the bacteria some of the solution was filtered through a bougie, and to some of it disinfectants were added. The filtration through bougies failed entirely. The filtrate free of bacteria no longer possessed in the slightest degree the dissolving power, while the unfiltered solution showed this in the greatest degree until the close of the experiment. It must be that the enzyme can not pass through the bougie or at least only in a very dilute solution. For this experiment 100 cc. bougies were used with Reichel's apparatus and 300 cc. of filtrate was obtained. Since the action of the enzyme, as will be shown later, is not essentially diminished by great dilution, it is certain that the filtrate was completely enzyme free. Potter and Laurent have been able to filter through bougies the solution containing the enzyme of their bacteria without impairing its activity. It must here be noted that the slime present in the juice of the plant decayed by our bacteria was also unable to pass through the bougie, so that the filtrate was no longer viscous. Perhaps the collection of this slime on the outside of the bougie made it impossible for the enzyme to pass through. Neither is it possible even with very dilute juice to obtain active filtrate.



A very easy method of obtaining the enzyme in large quantity in a solid state is by precipitation from the juice of decayed plants with 5 to 6 times the quantity of absolute alcohol. By this method the slime, swollen in the fluid, is precipitated in white particles which contain the enzyme and the bacteria. The precipitate is purified by 5 or 6 decantings with alcohol allowed to stand for a day in ether, changing the latter often, then filtered and dried. There results a gray white mass, easily breaking up in a mortar to a fine powder which, when water is added, swells up again to a viscous fluid, the latter when filtered furnishes a very clear filtrate with only a few dead bacteria. When this filtrate is used in the same dilution as the original juice its dissolving power is as great as that of the latter. This enzyme solution, whether from potatoes, carrots, or onions, is neutral to litmus. The activity of the dry enzyme powder is not diminished after it has been kept for four months. A quicker way of obtaining this enzyme solution is by the addition of a disinfectant to the juice to check the action of the bacteria without essentially diminishing the power of the enzyme. The substances tried were ether, chloroform, cyanide of potash (2 per cent), mercuric chloride (0.1 and 0.2 per cent) and formalin (0.1 and 0.2 per cent). The most satisfactory are formalin and chloroform, a 0.2 per cent solution of the former kills the bacteria without noticeably diminishing, at least in the beginning, the action of the enzyme. A 0.1 per cent solution does not always produce a sterile solution. Chloroform is usually as sure as 0.2 per cent formalin, and has no effect on the enzyme action at the expiration of a longer time. On the other hand ether only restricts the bacteria in their development without killing them. Potassium cyanide was less sure in its effect. One-tenth per cent mercuric chloride did not always kill all the bacteria; 0.2 per cent was safer, although resulting, especially in the potato juice, in the throwing down of large quantities of quicksilver in the form of mercuric sulphide. Two-tenths per cent sublimate had a very injurious effect on the enzyme.

Juice which is kept for 24 hours without the addition of any disinfectant has a very offensive odor. In 3 days, however, it still has the same tissue-dissolving power as in the beginning. After 6 days this was very much diminished and in 15 it had vanished altogether. Juice to which chloroform and ether had been added, had at first the same dissolving power as that containing no disinfectant. Diminution of this power in the juice treated with chloroform was evident first after 15 days, while in that treated with ether there was no change, although the bacteria in the latter were not killed but only checked in development. A 0.1 per cent solution of formalin did not make the juice sterile; when 0.2 per cent was added, at the end of 19 hours the dissolving power was as great as in the juice not treated. At the end of 43 hours it was noticeably diminished, but at the end of 6 days it was greater than in that of the juice not treated; at the end of 15 days it was very slow but still evident. One-tenth per cent solution of mercuric chloride did not kill the bacteria, but clearly restricted their development. At the end of 19 hours the action of the enzyme was essentially weakened then gradually in the solution which contained sulphur compounds the mercury was thrown down as mercuric sulphide, and the solution showed at the end of 15 days a renewed strong action. Two-tenths per cent sublimate killed the bacteria but weakened very much from the beginning the action of the enzyme, although at the end of 15 days the latter was not completely destroyed.

In another series of experiments in which the disinfectant was added to a solution free of bacteria that is, to the alcoholic precipitate of potato juice and water, the result was the same. At the end of three weeks the solution which was kept without any disinfectant and those treated with ether and with chloroform showed very powerful enzyme action. This was also true at the breaking off of the experiment two weeks later. Solutions treated with 0.1 and 0.2 per cent formalin and sublimate had at the end of three weeks only very weak dissolving power; at the end of 5 weeks this was completely lost in the solution treated with formalin.

To test the dissolving power of the enzyme on the middle lamella in tissues from various sources, thin razor sections were used. These were immersed in the enzyme solution and from time to time examined under the microscope; they were also tested by a light pressure on the cover glass.

The potato juice acted almost twice as quickly as the onion juice while the action of carrot juice was intermediate. In general, thin sections of carrot immersed in potato juice were completely broken up in ten minutes—20 minutes at the latest—so that only with difficulty could they be lifted out of the fluid, and the slightest pressure on the cover glass broke them up completely into individual cells. With the onion juice this result was obtained in 30 to 40 minutes. Neutralized sap worked no better, so that the poorer dissolving power is to be attributed to the less quantity of the enzyme, since the growth of bacteria on onion is less luxuriant and rapid than on potato, as the result of the greater acidity of the former. These sections, when examined under the microscope, showed the same phenomena described as occurring in the diseased plants: middle lamella dissolved, cells separated one from another, membrane somewhat thicker in the cells of the diseased portion than in normal tissue, outer contour indistinct. Further changes of the membrane after a longer immersion in the enzyme solution have never been observed. The membrane always gave the cellulose reaction.

Since the relation of the middle lamella to the dissolving enzym is of the greatest importance to bacterial infection, tissues from different sources and from plants of different ages were tested. Thin sections were immersed in an enzym solution from decayed potatoes and carrots. The plants tested were potatoes, carrots, onions, roots of yellow turnips, red beets, apples, seeds of *Phaseolus vulgaris* green and ripe, the pods of *Phaseolus vulgaris* both green and dried, date seeds, stem of *Phaseolus vulgaris*, *Vicia faba*, *Cucumis sativus*, *Zea mays*, *Avena sativa*, *Brassica acephala*, young and old plants, stem and leaf stalks of *Solanum tuberosum*, and *Lycopersicum esculentum*, leaf stalks of *Daucus carota*, *Petroselinum sativum* and *Apium graveolens*.

The results were as follows: At the end of several days' immersion in the solution the stems of maize and oats, and the endosperm of date were entirely unchanged. The breaking up of the tissue of red beet was extraordinarily slow. At the end of 60 hours the first evidence of softening was observed in the thin sections and it must for the present be considered a question whether a complete breaking up of the tissue can occur. Of all the other plants tested, the parenchyma tissue was completely broken up into single cells with unchanged cell-membrane, while on the other hand all the suberized and woody tissues were unaffected by an immersion of long duration in the enzym solution. The youngest tissue was broken up most rapidly, thus sections of the green seeds of *Phaseolus vulgaris* were disintegrated in about 20 minutes; those of the ripe ones in about 2 hours. Green pods of the same plant were broken up much more quickly than those completely dried ones gathered in the late fall. Likewise the parenchyma of stems of *Brassica acephala* was disintegrated more easily in June than at the end of November.

Experiments were then carried on to test the action of acidified juice from decayed plants sterilized with formalin, on the tissues of a single plant, *i. e.*, the carrot root. Acidification was made with malic, tartaric, and hydrochloric acid. The juice was mixed with the acid, left standing 4 hours at room temperature, 18 to 20°, and then tested as to its dissolving power. Potato juice was always used. This titrated + 5 to phenolphthalein.

The action of the enzym is gradually diminished by the acidity of a solution titrating + 10, and it entirely ceases when the solution titrates over + 20. Citric acid and hydrochloric acid have the same effect while malic acid is seemingly not as effective.

The enzym, even in a very weak dilution remains active. The rapidity of a solution of the tissue decreases proportionally to the amount of dilution of the enzym solution.

The power of the enzym to destroy the middle lamella is destroyed by boiling. A much lower temperature is also sufficient for this. Experiments show that the action of the enzym is destroyed if kept at 60° for any length of time.

According to the investigations of various experimenters the middle lamella is composed of the calcium salt of pectic acid. Experiments were carried on to show whether this could serve as a nutrient substance for the bacteria. Calcium pectate from carrots and yellow beets to which was added various nitrogenous substances was inoculated with the bacteria. A nutrient solution containing 2 grams of  $\text{KH}_2\text{PO}_4$  and 1 gram of  $\text{MgSO}_4$  to the liter was used. As sources of nitrogen 1 per cent asparagin, peptone, potassium nitrate and ammonium phosphate were added. If the medium was not neutral it was neutralized with soda,—temperature 28°. The flask containing the saltpetre showed no growth. On the contrary, in all the others there was very evident growth in 48 hours. The gelatinous content of the flask cleared in the upper portion to a thin liquid, which was sharply differentiated from the ever diminishing pectate layer. In 14 days the flask containing ammonium phosphate was slightly cloudy throughout and the contents were changed into a thin fluid with a small amount of precipitate. The fluid had a slightly aromatic odor, was weakly alkaline and very quickly dissolved into their elements sections of carrot treated with 0.2 per cent formalin. The solution of the calcium pectates was somewhat slower in the flask containing the asparagin, and still slower in that containing the peptone. The contents of the control flasks remained gelatinous. The fluid in the flask containing the ammonium salt gave the following reactions:

Absolute alcohol.....	No precipitation.
Dilute hydrochloric acid.....	No precipitation.
Alcohol + hydrochloric acid.....	No precipitation.
Boiled with Fehling's solution.....	No reduction.
Boiled with concentrated hydrochloric acid.....	Furfural reaction.
Boiled with concentrated hydrochloric acid and with Fehling's solution.....	Very strong reduction.
Slightly heated with dilute caustic potash lye.....	Yellow color.

On evaporating, a brown horny mass remains. The solution then contains a body, one of the pentoses—perhaps one of the hexose group—which is distinguished from calcium pectate only by its solubility. It is thus probable that the calcium pectate has been converted into metapectate by the enzym of the bacterium. A further splitting up of the metapectate into sugar does not appear to take place outside of the bacterial cell. Alcohol is also present, apparently. At least the solution

always gives a very strong iodoform reaction. The precipitate remaining in the flask is dissolved in part by acetic acid with evolution of gas; and with ammonium oxalate an abundant precipitate of oxalate of lime is obtained. It seems then that in the fermentation of the calcium metapectate, a portion of the lime is used to form carbonate of lime. The solution still contains some lime. When the solution was acidified with hydrochloric acid only the least trace of substance could be extracted with ether. No considerable acid fermentation, such as occurs in sugar solutions inoculated with the bacteria, takes place in the pectate flasks, since the liquid always remains neutral.

Thus the part played by the enzyme in the parasitism of this organism is as follows: It makes possible a rapid penetration to the deeper plant tissues of the poison secreted by the bacteria; by the resulting death of the protoplasm the cell-sap which is rich in nutrient substances is made accessible to the bacteria, insuring their more rapid multiplication, so that the plant is unable to bring its natural means of protection into play quickly enough. On the other hand, the middle lamella furnishes a suitable source of carbon to the organism, and finally the carbonate of lime liberated in the fermentation of the calcium pectate and perhaps also of the pectates themselves combines with the acids which are always produced by the bacteria in fluids containing sugar, and which would otherwise restrict the development of the organism.

Further experiments were carried on as to the manner of the poisoning of the protoplasm of the plant cell by the bacteria. If sections of carrots immersed in the expressed juice of rotted potatoes or carrots to which no disinfectant has been added, are observed under the microscope, one sees in ten minutes a slow separation of the protoplasm from the cell-wall the former at the same time becoming plainly granular. This process takes place slowly. In 30 minutes the protoplasm is still further contracted and apparently dead; in 40 minutes it is rolled up into a ball, deformed, dark brown, dead, in the middle of the cell. The same phenomena occur with sections of potato, onions, and cattle beets (Futterrüben).

If the juice from the decayed plants is boiled, its poisonous action seems to be completely lost. When sections of carrot are immersed in it nearly all the cells are living after 36 hours, as is shown by the production of plasmolysis with salt-peter solution. However, one finds that in such sections some of the surface cells are dead and the protoplasm therein shows the same phenomena as that in those sections which have been immersed in the fresh juice. Furthermore, it appears that the poisonous substance diffuses through membranes only with the utmost difficulty. Thus filtrate from juice which has been passed through a bougie is usually at first not at all poisonous, and only slightly poisonous after the filtration of 400 cc., being similar in this respect to boiled juice. And the solution of the middle lamella and the action of the poison go hand in hand as can be easily observed under the microscope. The poisoning never precedes. If one uses sections which vary in thickness, then one sees very plainly that plasmolysis sets in at the same instant that the cells are separated from one another by the solution of the middle lamella. The sound united cells and the diseased cells which are becoming separated are sharply differentiated. The poisonous action never extends beyond the decayed area, as is so often observed in fungous diseases.

There is apparently no labile toxin present. No oxalic acid is present, at least not in the neutral to alkaline potato and carrot juice, and it was not detected in the acid fermentation produced by the bacteria in sugar, glycerin, and mannitol solutions, a test which excludes free oxalic acid and various of its compounds.

The poison also is precipitated from the juice of decayed potatoes, carrots, and onions, together with the slime and the pectine dissolving enzyme, by the use of alcohol. If one dissolves the precipitate in an equal amount of sterile water, one obtains a neutral solution which in the activity and manner of its poisoning differs in no way from the original juice. On boiling a granular precipitate is obtained and the boiled solution still retains after many days a slight poisonous action on the protoplasm.

The most recent extensive contribution to the subject is by L. R. Jones (1910). A summary of this paper follows:

A detailed study was first made of the enzyme produced by *Bacillus carotovorus*, both living cultures and the enzyme isolated from them being used in the experiments. Later, comparative studies were made with enzymes secreted by other soft-rot organisms, other classes of bacteria, fungi and germinating seeds.

The same strain of the carrot-rot organism was used throughout. It is one isolated from decaying carrot tissues in 1899 and since\* grown on artificial media (practically all of the time in beef broth), at room temperature 16° to 22° C. Jones believes that there has been a considerable decrease in pathogenicity accompanied by a corresponding decline in the amount of enzyme production.

\*The studies here reported upon were carried on during the years 1901-1904.

Five methods were used for the isolation of the enzyme: (1) heat, (2) filtration, (3) germicides, (4) diffusion through agar, (5) precipitation by alcohol.

(1) Cultures 7 days old were immersed for 10 minutes in the water bath at 55° C. (51° C. thermal death point of the organism). Blocks of living carrot tissues were cut under sterile conditions and put, some into the heated tubes, some into living beef-broth cultures, and some into sterile uninoculated broth.

*Result.*—Tissues rapidly softened and fully decomposed in 3 or 4 days in the living cultures; a similar softening and complete decomposition in 10 days in the heated tubes; no softening in the sterile, uninoculated broth. Similar results were obtained with turnip root and cotyledons of immature peas. Heating at 54° to 62° C. inhibited the action of the enzyme, and temperatures above 63° C. (also at 62° and 63° C. with one exception) checked it entirely.

(2) A sterile solution was obtained without difficulty by means of the Chamberland filter. Broth cultures, varying in age from 7 to 14 days, were used. In all cases the middle lamella in sterile blocks of fresh roots of carrot and turnip, potato tubers, and the cotyledons of young peas immersed in the filtrate was dissolved as in the presence of the living organisms. In one experiment a piece of carrot about 3 mm. in diameter was perceptibly softened in 24 hours and softened throughout in 3 days, while potato blocks of the same size showed the first signs of softening in 5 days.

Comparisons were made of the enzyme activity of filtered sterile broth, unfiltered broth cultures and cultures of broths sterilized with chemicals. In one experiment thin razor sections from roots of carrot and turnip were immersed in (a) culture broth sterilized by filtration, (b) culture broth sterilized by a 20 per cent addition of chloroform, (c) solutions of alcoholic precipitate from culture broth. The solutions (b) and (c) acted about alike, whereas (a) required at least twice as long to disintegrate the tissues.

Similar trials made using razor sections of turnip, showed that the enzyme-action in sterile broths like (b) and (c) was practically the same as in living cultures. Some of the experiments indicate that possibly four-fifths of the enzyme was lost by filtration through the porcelain.

All these experiments show that passage through Chamberland filters removes a large proportion of the enzyme-content of the broth. The reason for this has not been determined. Freudenberg found that although all the bougies used by him retained considerable of the nitrogenous matter a new filter retained much less than the same one after it had been used several times. Later he found that the enzyme galactase was removed from milk passed through these filters.

Potter found that the enzyme produced by *Pseudomonas destructans* passed through a Chamberland filter and Laurent found that similar bacterial enzymes were not removed by filtration through porcelain. Spieckermann, on the other hand, obtained contrary results, the culture broths which he had filtered through a Reichel porcelain filter having not the least enzymic action. Van Hall found that the juice from potato decayed by *Bacillus subtilis*, when passed through the porcelain filter was still capable of rapidly destroying potato tissue. The juice from iris invaded by *Bacillus omnivorus* when passed through a porcelain filter retained its enzymic activity but in a less degree. In other trials he found that filtered broths lost all enzymic activity. Jones is at a loss to reconcile some of these results with his own, except by attributing the discrepancy to differences in the filters.

(3) Trial was made of the addition of formalin, phenol, thymol, and chloroform, respectively, to beef broth cultures.

*Formalin.*—Both the organism and the enzyme are extremely sensitive to this chemical. However it is possible to use an amount which will sterilize the broth and leave the enzyme active. The following conclusions have been reached:

One-tenth per cent of formalin sterilizes a beef-broth culture of *B. carotovorus*, one to ten days old, provided that the tube is thoroughly shaken. Otherwise more formalin, 0.2 per cent or more, may be necessary. Enzyme action is completely inhibited by 0.6 per cent of formalin and even 0.3 per cent retards to a marked degree, while there was perceptible retardation from 0.06 per cent formalin, although this amount was too small to insure sterilization. In all the above trials several days elapsed between the addition of the formalin and the trial of enzymic activity.

Similar results were obtained when tests were made to determine the effect of formalin in solutions of the enzyme obtained from broth cultures by precipitation with alcohol.

Spieckermann reports that a 0.2 per cent solution of formalin sterilized the cultures of the soft-rot organism of cabbage with which he was working without inhibiting the action of the cytolytic enzyme, at least for several hours. Jones undertook to learn the relative rate of action of formalin upon both *B. carotovorus* and its enzyme. Broth cultures to which 0.2 per cent of formalin had been added were shaken thoroughly and tested at frequent intervals both for viability and enzyme-action. The results though varying considerably, agreed in showing that the action on the organism is more rapid than on the enzyme. There was no appreciable effect on the enzyme action for from 3 to 9 hours, or, in one case, 24 hours, whereas there was marked inhibition to growth of the organism after 2 or 3

hours. The use of formalin, however, is not a practical method of sterilizing broths preparatory to the study of the normal action of the enzyme, since sterility is not assured by 2 or 3 hours subjection to formalin and if a longer time elapses the activity of the enzyme is affected.

Bliss and Novi have shown that proteolytic enzymes have no effect upon fibrin, which has been acted upon a short time by formalin. This raised the query whether retardation in the cytolytic action is not due to the action of the formalin on the cell-wall of plant tissues rather than upon the enzyme itself. Experiment proved that this was not the case. Sections immersed 24 hours or even a month in formalin or absolute alcohol were decomposed by the enzyme solution as quickly as fresh sections. Bliss and Novi found that formalin inhibited certain enzymes (papain, trypsin, amyllopsin) and not others (pepsin, malt diastase) and von Freudenreich discovered that formalin tends to lessen the action of galactase more promptly than it does that of pepsin and pancreatin.

*Phenol*.—An addition of 0.3 per cent or 0.6 per cent of phenol, if the culture was well shaken, always produced sterility, and there was no apparent retardation of the activity of the enzyme; 0.1 per cent failed to sterilize, and 5 per cent totally inhibited the activity of the enzyme. The phenol was allowed to act four days before the enzymic activity was tested.

*Thymol*.—This was less effective than phenol, probably because of its slight solubility and slow diffusion in the broth. A small amount will kill the organism if the cultures are shaken thoroughly but even large amounts fail to sterilize in the absence of agitation. There is no evidence of any inhibition of the enzyme action.

*Chloroform*.—From 10 to 50 per cent of Powers & Weightman's chloroform, "U. S. P. Standard," added to cultures 7 to 9 days old failed to kill the organism in 3 days, but when the experiment was repeated 2 years later, using both Mallinckrodt's "M. C. purified" chloroform and the U. S. P. grades both of this firm and of Powers & Weightman, sterility was secured in every case. The enzyme action was not affected.

Brown and Escombe report that the cytolytic enzyme of barley is not appreciably affected by a saturated aqueous solution of chloroform. Smith found that many organisms are surprisingly resistant to chloroform and emphasizes the need of caution in its use. Potter did not obtain sterility in his cultures of the turnip white-rot organism with chloroform but Spieckermann succeeded in sterilizing the sap of vegetables invaded by his kale-rot organism by the use of it. There was no appreciable retardation of the cytolytic action with the exception of a possible gradual weakening after 15 days or more. Van Hall's results with *B. omnivorus* are surprisingly at variance with these since he found the addition of even 0.5 per cent chloroform destroyed all trace of activity in bacterial juices in one quarter of an hour.

A special series of these experiments was planned to demonstrate the comparative effect of these chemicals on the activity of the enzyme. These confirmed the evidence of previous trials and led the author to conclude that in the doses named neither chloroform, thymol, nor phenol caused any diminution of the cytolytic action; that formalin inhibited the enzymic activity; that filtration through porcelain reduced the enzyme content.

(4) Observations upon decaying vegetables have shown that cytolytic action goes on some distance in advance of the invasion of the organism. Experiments were carried on to test the diffusion of the enzyme through some medium impenetrable to the bacteria. Small Petri dishes containing 2 per cent beef broth agar about 3 mm. deep, were inoculated in the center with *B. carotovorus*. In 3 days there was a good surface growth, about 1 cm. in diameter. A slice somewhat larger than this layer of agar, was then cut from the interior of a fresh turnip root and placed in a large sterile Petri dish. The layer of agar from the smaller dish was then placed upon the surface of the turnip, care being taken to avoid contamination. At the end of 24 hours the turnip showed an area immediately underlying the colony and somewhat larger than the same in which the tissues were slightly brown and softened exactly as though invaded by the organism. Bits of this rotten turnip tissue were transferred to each of three broth tubes but in none of these did growth develop, proving that this softening of the tissues was due solely to substances secreted by the bacteria which diffused through the layer of agar from the surface colony above. Microscopic examination showed isolation of the cells as a result of the solution of the middle lamella, a swelling of the residual walls, and granulation and plasmolysis of the cell-contents. There was no softening in the check.

(5) By the use of strong alcohol a flocculent whitish precipitate is obtained from broth cultures. This includes the enzymes, various proteid matters and also the bodies of the bacteria. This dry precipitate can be preserved indefinitely, as the use of strong alcohol insures the elimination of the living organism, 25 per cent being fatal to *B. carotovorus*.

It was found preferable not to pass the broth through a porcelain filter, as the filtered broth possesses less of the enzymic activity and, therefore, as repeated observations had shown that this enzyme is entirely inactive on the celluloses proper, the culture broths were passed through filter paper. The succeeding steps were as follows:

"To the filtrate was added enough 95 per cent alcohol to render it alcoholic to the desired degree, usually 80 per cent. The precipitate allowed to settle, the supernatant alcohol syphoned off, the precipitate collected on filter paper, washed with either absolute or 95 per cent alcohol and quickly dried, partially in a current of warm air, then in a desiccator over sulphuric acid. The dried precipitate, which is gray and somewhat brittle, was then powdered before redissolving in water. It is of course important to secure quick drying to avoid the possibility of alteration as a result of bacterial growth or of chemical changes in the precipitate. The drying must also be done at so low a temperature as to preclude danger of injury from heat to the sensitive enzyme."

Tests of different per cents of alcohol showed that 80 per cent secured practically all of the enzyme and that the latter was in a state of the highest activity. Re-precipitation proved to be of little advantage. The activity of the enzyme increased with the strength of the solution, but not proportionally. After several trials the author came to the conclusion that, with the precipitate used, it required on an average, 25 minutes for a 1 per cent solution of the precipitate to secure as complete enzymic action as was secured in 15 minutes in the 5 per cent solution, and in 10 minutes in the 10 per cent solution.

In all the work reported here 5 per cent solutions have been used unless otherwise stated.

Comparative tests showed that no loss of cytolytic activity occurs through precipitation and re-solution. On the other hand, the 5 per cent solution of the precipitate rotted the vegetable solutions in less than one-third the time required by the living cultures (5 per cent solution of the precipitate, however, contains about 15 times as much of the enzyme as the broth cultures).

Jones found the relation of cultural conditions to enzyme production to be as follows:

*The Medium.*—The vigor of growth of the organism varies widely with the medium, age and temperature. Various experiments were undertaken to determine the relation of these factors to enzyme formation, some previous investigators having concluded that enzyme production is in some cases a starvation phenomenon.

The media used were (1) Dunham's peptone solution = a very weak growth; (2) the same + 2 per cent cane sugar = twice as dense growth as in the simple Dunham's solution; (3) neutral beef broth = good growth; (4) the same + 2 per cent cane sugar = more rapid growth than in plain broth; (5) cooked carrot broths — (a) those in which equal weights of pieces of fresh carrot roots and water were cooked and sterilized together by discontinuous sterilization, and (b) the same in which after the first cooking in the steamer the cell-wall substances were removed by crushing the roots and filtering through several thicknesses of paper. This filtrate was then sterilized in the steamer by the fractional process. Both of these have, except in certain cases, proved to be the most favorable cooked media for this organism; (6) living vegetables—this class of media has given the greatest and most active enzyme product. Beef broth yields on the average about 0.25 per cent of dry precipitate while expressed juice from decayed turnip after filtration through paper, has yielded over 0.5 per cent precipitate, a 5 per cent aqueous solution of which caused the complete rotting of the razor section of turnip in 10 minutes, whereas a like solution of the precipitate from a beef broth culture required nearly 2 hours. Thus not only was twice as much precipitate obtained from the decayed living vegetable but the solution was 12 times as active.

When sections were immersed directly in the living cultures, *i. e.*, in the juice from decaying turnip, and in beef broth cultures, those in the former were rotted 2 or 3 times as quickly as those in the latter. The presence or absence of cell-wall substances, the effect of which was tried in the unfiltered and filtered vegetable broths, makes no difference in the enzyme production. The cooked vegetable media, however, were not uniformly satisfactory. In some cases there was excellent growth, in others very little. The enzymic development was directly proportional to the amount of growth. Jones used beef broth cultures largely in his comparative studies because more reliance could be placed upon the uniformity of growth in this medium.

The addition of 2 per cent sucrose causes a more vigorous growth of the organism especially in the earlier stages. More precipitate and more enzymic activity were developed in the sugar broth than in the plain broth. In conclusion, then, the author notes that the amount of enzyme developed seems to be directly proportional to the rate and vigor of growth; that the presence of cell-wall substances have no appreciable effect on the amount of enzyme developed; and that in beef broth cultures the addition of sugar favors growth and increases the enzyme production. Enzymic production in this case at least is not a starvation phenomenon. The experiments with filtered and unfiltered vegetable broths indicate that the organism makes little or no use, for nutrition purposes, of the cell-wall substance which it dissolves.

*The Age of the Culture.*—The enzyme content in carrot broth cultures increased with the age of the latter. Cultures were grown at 20° to 22° C. and tested at the end of 1, 5, 3, 5, 7, and 9 days. Between 1, 5 days and 5 days there was a rapid increase in the enzymic action (scarcely distinguishable in 1, 5 days, but very pronounced in 5 days). From the fifth to the ninth day it continued to increase but at a slower rate. Beef broth cultures tested at the end of 4, 6, 9, and 18 days gave

similar results as did also 5 per cent solutions of precipitates from beef broth cultures 3, 6, 9 and 17 days old.

*Temperature.*—The optimum temperature for most rapid growth of *B. carotovorus* is 28° C. to 30° C. Contrary to expectations, however, enzym production is not the most active at this temperature. The enzym content of broth cultures grown for 8 days at room temperatures (18° to 22° C.) was greater than that of similar cultures grown the same length of time at 30° C.

The activity of the enzym with respect to the following environmental conditions was also determined:

*Effect of Long Keeping.*—In the author's opinion there was no loss of enzymic activity in dried enzym-containing precipitate kept for months or even years.

*Relation of Temperature to Activity.*—Solutions of the alcoholic precipitate from carrot broth cultures were tested on carrot sections at different temperatures. Action slight at 2° C., good at 22°, better at 32°, best at about 42°, inhibited somewhat at 48°, pronounced inhibition at 50°, practically complete inhibition at 51° and above. The optimum lay between 40° and 45° C. Such solutions were uninjured by an hour at 49° C. either in the presence or absence of carrot tissues, but practically destroyed by exposure to 51° for 10 minutes.

Comparative studies show that the points of inhibition and destruction were approximately 10 degrees lower in the solutions of the precipitate than in the original broth.

In studying the effects of acids and alkalies the alcoholic precipitate obtained from carrot broth cultures was used. The strength of the acid and alkali solutions was determined by titration with phenolphthalein.

*Alkali.*—The presence of sodium hydroxide, titrating — 2 per cent, inhibited the reaction slightly. When sufficient alkali was added to make the liquid titrate — 10 per cent, inhibition was total.

*Acids.*—(These acids were made up by weight and titrations of strength determined afterward).—A very small amount of hydrochloric acid seemed favorable to the action of the enzym, a reaction of + 0.5 per cent being about the optimum. There was great inhibition when the reaction was + 2 per cent and at + 5 it was practically complete.

Various organic acids were tested, the results in detail as given by the author are shown in the following table:

Acid.	Strength of titration.	Effect.
	<i>P. ct.</i>	
Oxalic.....	+ 0.8	Retarded slightly.
Oxalic.....	+ 1.1	Retarded greatly.
Oxalic.....	+ 8.0	Complete inhibition.
Acetic.....	+ 0.2	No effect.
Acetic.....	+ 0.5	Do.
Acetic.....	+ 1.0	Retarded greatly.
Acetic.....	+ 10.0	Complete inhibition.
Formic.....	+ 0.15	No effect.
Formic.....	+ 0.4	Do.
Formic.....	+ 0.75	Retarded greatly.
Formic.....	+ 7.5	Complete inhibition.
Tartaric.....	+ 0.14	No effect.
Tartaric.....	+ 0.55	No effect (possibly slight retardation).
Tartaric.....	+ 5.5	Almost full inhibition.
Malic.....	+ 0.2	No effect.
Malic.....	+ 0.8	Do.
Malic.....	+ 8.0	Almost full inhibition.
Citric.....	+ 0.2	No effect.
Citric.....	+ 0.8	Do.
Citric.....	+ 8.0	Almost full inhibition.

"From these results it will be seen that these organic acids in no case aided the action; that where the acidity, as shown by titration, was + 0.5 per cent and less they were practically without effect; that + 1.0 per cent and above distinctly inhibited in all cases where it was tried, and that from + 5 per cent to + 10 per cent led to complete inhibition."

*Effect of Plant Juices.*—Some experiments were carried on to determine whether the normally acid cell sap has a similar effect on the enzym. To the expressed juice of carrot, radish, and ripe tomato, were added equal parts of a 5 per cent aqueous solution of the precipitated enzym from a carrot broth culture. There was a slight diminution in the rate of action as compared with solutions in distilled water. This was a little more pronounced in the case of the tomato. The test was repeated

with the tomato juice by adding 5 per cent of the dried precipitate directly to the juice. Here the retardation was nearly one-half. These vegetable juices were titrated and the acidity shown to be as follows: tomato, +5 per cent; carrot, +2 per cent; radish, +0.75 per cent.

*Effects of Other Bacterial Products.*—A small amount of some undetermined acid is produced by this organism in the presence of carbohydrates. In order to determine the effect on the enzymation of this or other products of the bacterial metabolism, broths of various kinds in which the organism had been grown, were sterilized and their enzym content destroyed by heating to 80° C. To two parts of each of these heated broths was then added one part of a water solution of the precipitate containing the enzyme, and a comparison was made of the activity of these mixtures and that of a solution of like strength of the precipitate in pure water. There was more or less inhibition in the broths in every case. The evidence, therefore, is that the products of bacterial metabolism inhibit rather than aid the cytolytic action of the organism.

There is no diastasic action worthy of note, although a slight tendency to the extremely slow conversion of starch into amyloextrin has been observed. No erosion of the starch grains takes place.

There have been many opinions regarding the composition and origin of the middle and inner-lamellae of cell-walls. Comparatively recently it has been shown that the "cellulose," of which it has long been known that parenchymatous walls are composed, includes a group of closely-related compounds. Moreover, the middle lamella does not give the cellulose reactions and the inner lamellae contain other substances in addition to cellulose.

Cross and Bevan (1895), in their work on celluloses make two groups, (1) the cellulose group, (2) the compound celluloses. The cellulose group is further subdivided as follows:

- (a) Resistant to hydrolysis, e. g., cotton.
- (b) Less resistant to hydrolysis, found in grass stems, etc.
- (c) Low resistance to hydrolysis, found especially in fleshy roots and in seeds.

Groups (a) and (b) are termed the celluloses proper, group (c) "pseudo-cellulose" or "hemicellulose," the name used by Schultz. By "hemicelluloses" are meant "substances closely resembling in appearance the true celluloses but easily resolved into simpler carbohydrates by the hydrolytic action of an enzyme or of the dilute acids or alkalis."

The compound celluloses are divided into three groups:\* (1) "Pectocelluloses" of which the middle lamella is composed, (2) "lignocelluloses" which we commonly know as "wood," (3) "cutocelluloses" constituting the protective outer layer "cutin."

Of the compound celluloses those termed "pecto-celluloses" by Cross and Bevan, constitute the middle lamella and the other wall elements upon which the carrot rot enzyme acts. It was Fremy (1840, 1848), who found in plant cell walls, along with cellulose, another substance which he called pectose, and he also isolated from certain plant tissues (carrot roots among them) an enzyme capable of gelatinizing this pectose and related compounds. This enzyme he called "pectase." Fremy's observations and conclusions have been confirmed by chemists, and the pectose series of compounds is classed with the celluloses as Cross and Bevan's classification shows. Mangin's most extensive studies (1888-1893) prove that here again we are dealing not with a simple compound but a complex of closely related compounds. These he divides into two natural series, the one neutral, the other acid. Pectose belongs to the less soluble neutral series, and pectine is a more soluble form. Both of these are widely distributed, especially in the walls of young tissues. Pectic acid and its insoluble salt, calcium pectate, are of common occurrence and of peculiar interest to us. Fremy supposed that his enzyme, pectase, clotted the pectose solution by converting the pectose into pectic acid, but Bertrand and Malleuvre (1894, 1895) have proved that this clot is calcium pectate. Paven believed that the middle lamella is composed largely if not wholly of this salt and this belief has been confirmed by the recent studies of Mangin, and Bertrand and Malleuvre, who have also shown that the inner lamellae contain varying proportions of pectose or pectic compounds in addition to the celluloses.

Mangin's studies led him to conclude that the wall, in the early stages of its development consists for the most part, of the less soluble pectose, whereas later the calcium pectate predominates in the middle lamella, and the pectose which is present is in the inner lamellae, i. e., nearer the cytoplasmic layers. The proportion of cellulose becomes increasingly greater, however, as one passes farther away from the middle lamella. The splitting of the walls along the middle plane under the action of pectate solvents indicates the probable occurrence of a thin sheet of calcium pectate even in the young walls. This layer thickens and becomes more clearly defined until it is plainly visible in the mature cell as the middle lamella.

\*Cross, article on Cellulose, *Encyc. Britannica*, 11th edition, 1910.



Pectase is especially abundant in growing tissues, and is supposed to play an important part in this lamella formation by converting the pectose of the inner lamellæ into the more soluble pectine and ultimately into pectic acid, which then passes to the outer surface of the inner lamellæ where it combines with calcium and increases the middle lamella substance. The latter appears homogeneous, but is distinctly stratified in structure, at least at the angles of the cells.

Jones gives the following description of the appearance of attacked tissues:

"*B. carotovorus* rots only parenchymatous tissues. The invaded tissues become watery and usually more or less darkened in color when exposed to the air. The attacked cells rapidly lose all coherence and always show a sharply defined line of demarkation, indicating that the softening occurs quickly and completely after it begins. Examination of such recently decomposed tissues under the microscope shows the cells to be already isolated or easily separable along the plane of the middle lamella. The protoplasmic sac within the cell is collapsed, more coarsely granulated than normally, and evidently dead and in the process of disorganization. Bacteria teem around and between these cells but are so rarely seen within them that where this does occasionally occur, one is led to attribute it to mechanical rupture of the softened walls rather than to direct solution."

If a cut surface of root is inoculated and kept in an ordinarily dry atmosphere, the infected area dries out very rapidly, but if, on the other hand, it is kept in a saturated atmosphere drops of exudate teeming with bacteria form on the surface and the tissues underneath become sunken. Wilting or pithy and partially dried-out vegetable tissues of even the most susceptible varieties, such as turnip, radish, and carrot can not be infected by this organism. The areas actively invaded are the intercellular spaces and the planes of the middle lamellæ. An abundant moisture content in the host tissues is necessary for this invasion. The expulsion of gas caused by the filling of the intercellular spaces with liquid resulting from the plasmolysis of the cells, and the changes in the optical character of the walls themselves probably accounts for the water-soaked appearance of the invaded tissues. The walls are normally uniformly refractive throughout, but the inner lamella begins to lose its refractiveness almost immediately when it is immersed in a living culture of *B. carotovorus* or an aqueous solution of the precipitated enzyme. This change is evident to the naked eye if thin sections are used, and microscopical examination shows that it is associated with a swelling of the inner lamellæ, sometimes to twice their original thickness, this phenomenon being followed within a short time by a delicate laminated appearance of these swollen walls. The middle lamella also becomes less refractive, and the thinner portions soon begin to melt away. As these parts of the middle lamella dissolve, the heavier portions in the angles of the cells remain isolated. When this stage is reached, tapping on the cover glass will show that the cells have lost all cohesion. It requires from ten minutes to an hour for thin razor sections of carrot or turnip placed in living cultures or in active enzyme solutions to pass through these changes, although the complete solution of the thickest pieces of intercellular substance may not have taken place in this time. There is also a slight thinning of the inner lamellæ, but complete solution has never been observed, although the same sections have been under observation for three weeks. There was little change after the first few hours. The cellulose reaction is obtained even after the longest immersion. The lamination of the walls grows more apparent for a short time, but no further change takes place.

There is no action upon lignified or cuticularized walls. The solution of the middle lamella takes place considerably in advance of the invasion of the organism.

In the invasion of turnip and radish roots and cabbage petioles the conditions are similar to those found in the carrot but the rotting advances more rapidly. In the carrot the core rotted more quickly than the cortex tissues. The young potato became disintegrated more quickly than the mature tuber. On the beet root there was no solvent action whatever.

These studies indicate that aside from the moisture content, susceptibility to infection is largely, if not wholly, dependent on the nature of the middle lamella.

Comparative studies were made with 45 other strains of soft-rot organisms, including the following: Three strains of cabbage-rot bacilli isolated in Vermont by F. R. Pember in 1899; twenty-three other strains of cabbage-rot bacilli isolated in Vermont by W. J. Morse in 1901; one strain of turnip-rot bacillus isolated by L. P. Sprague in Vermont, 1903; twelve strains of soft-rot bacilli isolated by Harding and Stewart in New York, including one associated with the soft rot of *Amorophallus simlense*, and eleven from the soft rot of cabbage; six additional organisms, as follows: Townsend's calla rot, *Bacillus aroidææ*; Harrison's cauliflower rot, *Bacillus oleraceæ*; van Hall's two iris-rot organisms, *Bacillus omnivorus* and *Pseudomonas iridis*; Spieckermann's kale-rot organism (a *Bacillus*); and Potter's turnip-rot organism, of which the strain sent by Potter to Jones was also a *Bacillus*.

Comparative studies were made by Harding and Morse\* of forty of these forty-five strains, and three others, and their conclusion is that these forty strains probably constitute only one somewhat variable species.

Both living cultures and the alcoholic precipitates were used by Jones in these comparative studies. The experiments proved that these forty-five strains secreted the same middle-lamella-dissolving enzyme as *B. carotovorus*, and that, moreover, in these strains also complete solution of the cellulose and diastatic action are lacking.

After reviewing the literature the author states that he is convinced Green is right in his conclusion that the cytolytic enzymes fall into two natural groups, the one acting upon the pectic, and the other upon the cellulose elements of the cell-wall. Further knowledge regarding the chemical composition of the cell-wall will doubtless lead to a further subdivision of the enzymes acting upon it. At present we recognize in the cell-walls of the less modified plant tissues the following: 1. True celluloses. 2. Hemicelluloses. 3. Pectic compounds. In the more modified tissues there are compound celluloses, ligno-cellulose, etc.

There is evidence that there are enzymes which act upon the true celluloses but the cytolytic enzymes which have been studied in detail act only upon the hemicelluloses and pectic compounds. Believing these cytolytic enzymes to be as clearly separable into two groups as the elements on which they act, the author thinks it advisable that a distinct name be given to each of these two groups of enzymes.

The enzyme of *B. carotovorus* and of related soft-rot bacteria acts upon the pectic compounds, but not upon the hemicelluloses. Usually heretofore such an enzyme has been termed "cytase."

The author believes, however, that a more distinctive term should be applied to this class of enzymes, and inasmuch as the logical one of "pectase" has already been applied to Fremy's clotting enzyme, he favors the name *pectinase*, which was suggested by Bourquelot and Herissey. This term was originally applied to the enzyme which hydrolyzes pectose, but it was found later that this same extract hydrolyzes the coagulum, or pectic clot, and if, as seems probable to the author, this latter action is due to the same enzyme as the former, this name must be accepted for the enzyme under discussion. Jones gives the following definition of "pectinase:"

"Broadly defined, then, *pectinase* is capable of hydrolyzing pectose when in solution so that it will no longer yield a clot under the influence of pectase, and also of hydrolyzing the pectic coagulum and the pectic elements in the cell-wall, *viz.*, the middle lamella and parts of the inner lamellæ of certain tissues."

Bourquelot and Herissey did not state that their enzyme does not act on hemicellulose; in fact, hemicellulose is acted upon by barley malt solution with which their work was done. Taka-diastase acts predominantly on hemicellulose, although action on the pectic compound occurs also. Either two enzymes are present in taka-diastase or there is only one which is allied to "pectinase" but differs from it in its ability to act on hemicellulose. Newcombe's results strongly favor the first and exclude the second of these two possible explanations.

Believing that two enzymes are present in taka-diastase, barley malt, etc., pectinase and an enzyme acting upon hemicellulose—Jones suggests for the latter the name "hemicellulase." Thus the name "cellulase" could be used to include all cellulose enzymes, or, as the author believes preferable, reserved for enzymes acting upon cellulose proper.

\*Their studies did not include the following: Pember's R., *Pseudomonas iridis*, nor the New York organisms O. 1 II 6 c, O. 1 II 6 a, and the bacillus from *Amorphophallus*.

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## REACTION OF THE PLANT.

In many instances there is no perceptible evidence of defense or tissue-reaction in any part of the host, *i. e.*, the attacked plant succumbs quickly, offering no apparent obstacle to the advance of the bacteria. This is true of various soft rots, in virulent forms of pear-blight, in brown rot of young tobacco plants and tomato plants, and in the wilt of cucumbers. In less virulent forms of disease the plant often reacts by building a more or less impervious wall around the diseased parts, between them and the sound tissues, and thus "corks out" the intruder, *e. g.*, potato-tubers attacked by *Bacillus phytophthorus*, various leaf-spots, and cankers. In some instances the presence of bacteria in the tissues leads to the premature development of organs—blossoms and side branches in the squash, male inflorescence in sweet corn, clusters of roots from other roots in hairy root of apple, aerial roots on tomato, daisy (fig. 26), and tobacco; in other cases retardation of development and atrophy occur.

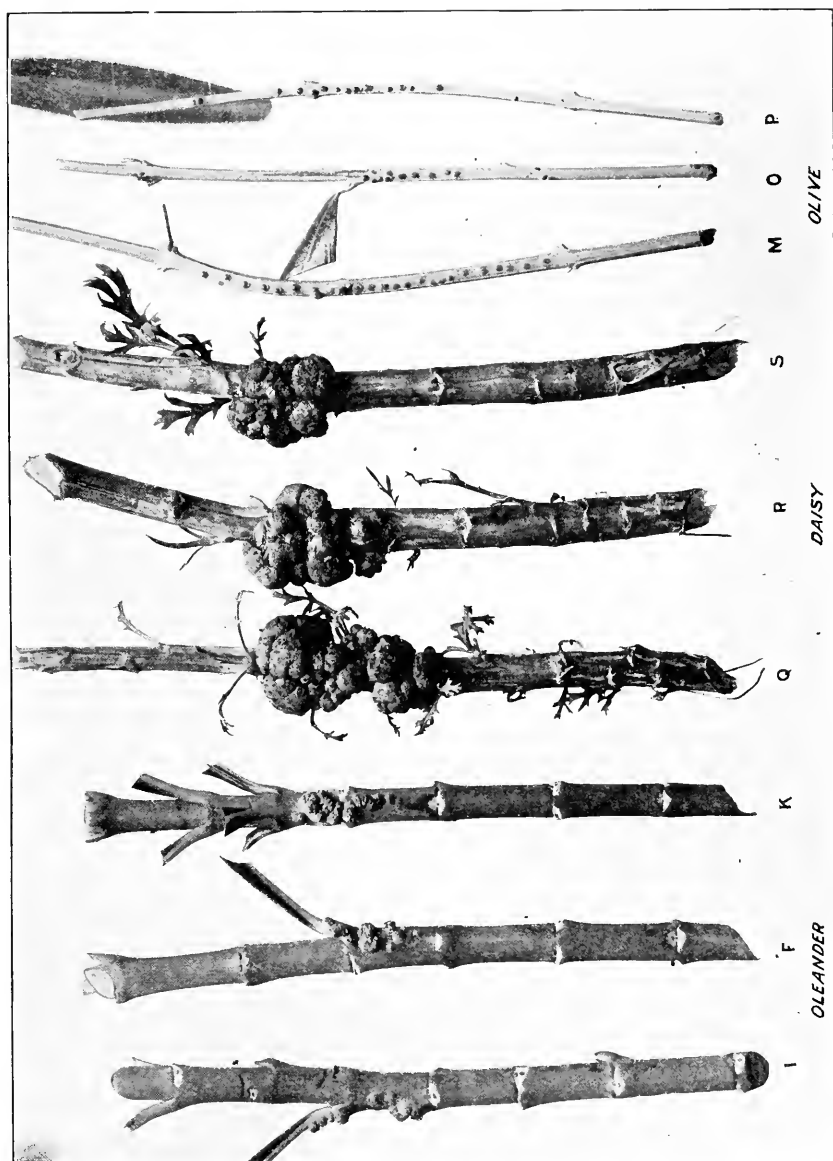
### HYPERPLASIAS.

In certain types of disease there is a very pronounced reaction of the host. This is manifested by rapid cell-division and enormous increase in the volume of tissues, the result being a tubercle or tumor which may continue to grow for months (plates 8 and 9) and exceptionally reach a diameter of a decimeter or more. The lowest stages of this hyperplasia may be seen in cankers of various sorts and in the effect of non-virulent cultures of *Bact. solanacearum* on potatoes and tomatoes (fig. 27). The most striking examples are the crown-galls of peach, hop, daisy, sugar-beet, etc. (figs. 28, 29). These enormous swellings are the result of repeated cell-division under the stimulus of the presence of the micro-organisms in the tissues and as already stated inside of the rapidly dividing cells. Just what this stimulus is we do not yet know. It is probably a definite chemical substance derived from the bacteria, *i. e.*, a by-product, or an endotoxin. The writer suspects ammonium or calcium acetate to be one of the stimulating substances. There is reason to suppose that acetic acid is formed by *Bact. tumefaciens* in tumors. Some substance liberated from the bacterial cells killed thereby may be the actual inciting cause. This whole subject is reserved for further consideration. Entomologists have maintained with respect to insect galls that if the egg is deposited anywhere but in the cambium layer, *i. e.*, too deep or too



Fig. 26.\*

\*FIG. 26 —Stem of Paris daisy showing incipient aerial roots at x induced by presence of tumor. This was inoculated by needle-punctures as a check on virulence of a culture of *Bacterium tumefaciens* inoculated into sugar-beets. Plant inoculated Nov. 15 (or 18), 1907. Photographed Feb. 20, 1908.



Crown Gall.

Stems of oleander, daisy, and olive two and one-third months after inoculating with daisy knot organism (*Bact. tumefaciens*) by means of needle-pricks. The oleander and daisy developed tumors the former slowly. The olive remained free. At end of seven months the largest tumors on the oleander were size of smallest on daisy here shown. Inoculated Mar. 12, 1906. Photographed May 24.



shallow, no gall results. In the crown-galls, growth may begin, it would seem, in the inner wood, in the cambium ring, in the outer bark, or in the mesophyll of a leaf, *i. e.*, wherever cells are naturally dividing. The division of cells may take place so rapidly that all or a large part remain small. The earliest stages of the tumor formation have not been traced in serial sections. Soon more definite information will be available.

In sections of young crown-galls mounted unstained in sterile water small clumps of bacteria may sometimes be seen inside of unbroken cells and moving granules and rod-shaped bodies in small numbers some of which have appeared to be self-motile, the longer ones flexuous and occasionally one constricted in the middle, but stained slides have thus far given no clear-cut pictures.

#### HYPERTROPHIES.

In hypertrophied tissues the individual cells are larger than normal. Usually both hyperplasia and hypertrophy occur in the same growth, *c. g.*, in olive-tubercle. Good examples of hypertrophied cells occur also in root-nodules of Leguminosae. Here their volume may become many times that of the normal cell. Hunger pointed out that tyloses are very common in the vessels of plants attacked by *Bact. solanacearum*, and ascribed their formation to the presence of the bacteria. Of the correctness of this view I have since satisfied myself. The writer has seen the same thing in the wood of young shoots of the mulberry attacked by *Bact. mori* (fig. 30). Here the stimulus to growth appears to be due to poisonous products absorbed by the vessels of the plant in advance of the movement of the bacteria. This is quite in accord with what we know of the action of many poisons, minute doses stimulating and larger doses destroying. The formation of tyloses in the manner described raises the question whether they may not be formed often under the stimulus of absorbed foreign substances, *c. g.*, in the roots of old cucurbits where they are very abundant. Various attempts were made by the writer to stimulate their formation in roots of young cucurbits by addition of ammonia and ammonium acetate but thus far with inconclusive results. The tyloses sometimes appeared within a few days, but small numbers of bacteria also occurred and may have been the determining cause. Only when we are able to obtain them promptly without contaminating bacteria can we be certain.



Fig. 27.\*

\*FIG. 27.—Swelling on a potato shoot inoculated with a non-virulent culture of *Bact. solanacearum* (Va.). Plant 118 inoculated Apr. 16, 1904. Photographed May 25. Disease at an end.

## ATROPHIES.

The olive-tubercle, due to *Bact. savastanoi*, affords an excellent example of atrophy. When the tubercle occurs on young stems that portion of the stem beyond the abnormal growth is robbed of a large portion of its nourishment and often dies outright. In less severe cases it is very common to find the length of a stem greatly reduced as compared with normal shoots of the same age and origin. The diameter of the stem immediately above the tubercle is also perceptibly less. Often it is not one-half or one-third as big as the stem immediately below the excrescence (see plate 9). Often also the leaves are dwarfed on that part of the shoot beyond the tubercle. Not infrequently when the terminal shoot becomes diseased in this way an inconspicuous side shoot takes the lead and becomes the strong shoot.

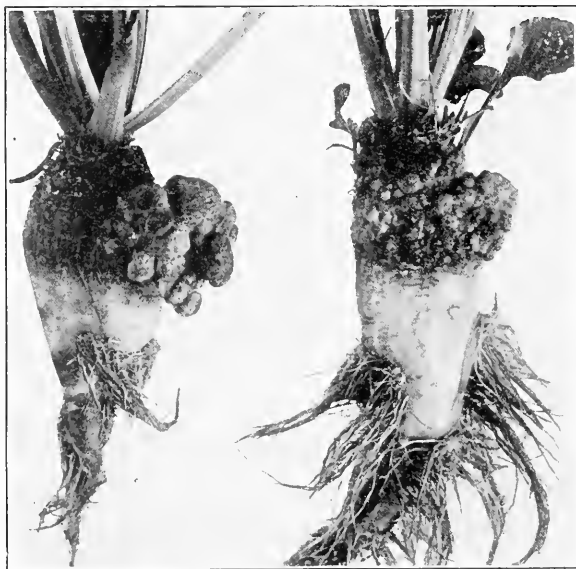


Fig. 28.\*

The same thing occurs in the daisy knot, due to *Bact. tumefaciens*. As growth of the knot continues, branches are often starved out and die (plate 10).

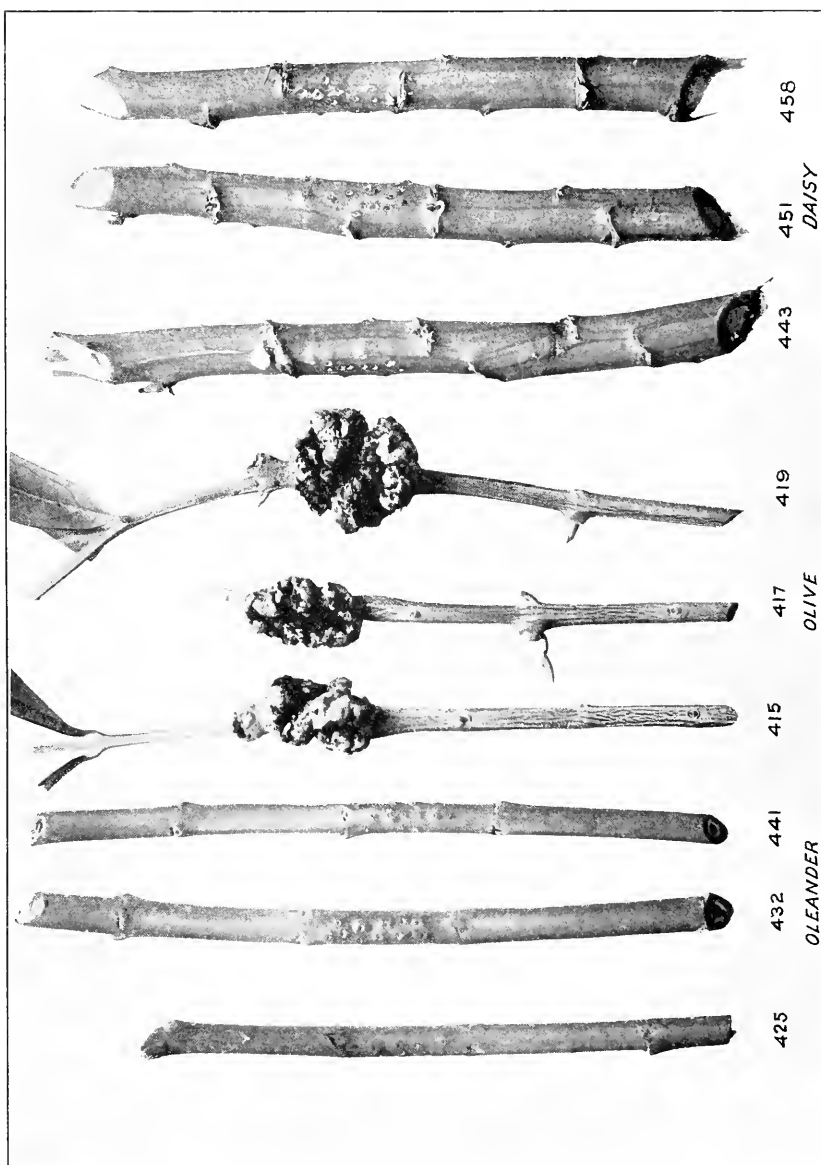
There is also a dwarfing of the whole plant in certain cases. This occurs very often in sweet corn attacked by *Bact. stewartii*, in cane attacked by *Bact. vascularum*, in seedling cabbages attacked by *Bact. campestris*, and in many plants attacked by crown-galls and root-galls.

## ENLARGEMENT OF THE NUCLEUS.

In many diseases which I have studied I have not been able to make out any change in the form or size of the nucleus, those nuclei near diseased areas being not different from more remote ones. The change in such cases, if any, is a simple disintegration under the action of the bacteria. In olive-tubercle, however, and in the crown-gall there seems to be

\*FIG. 28.—Tumors on sugar-beets due to inoculation by needle-pricks with *Bact. tumefaciens*, plated from Paris daisy. Inoculated about six weeks. Photographed April 1907. Nearly natural size.





Olive Tubercle.

Stems of oleander, olive, and Daisy four months after inoculating with olive tubercle. *Agrobacterium tumefaciens* (strain 104) by means of needle-pricks. All wounds on oleander and daisy plants healed without development of tumors. All inoculated olives produced tumors like those here shown. Inoculated Nov. 26, 1947. Photographed May 27, 1948.





Crown Gall.

Atrophy and death of a shoot of Paris daisy due to inoculation with *Bact. tumefaciens* by means of needle-pricks. Inoculated Dec. 1907. Photographed June 1908. About half natural size.



an enlargement of the nucleus, often to double the normal size, and often a change of shape to spindle form. In such tissues the nuclei stand out very prominently in the small cells, being the most conspicuous objects in the section. This size, however, may be a characteristic of extreme youth rather than of disease, since the writer has also seen large nuclei in the tissues of the growing point of healthy daisy-plants. The disorganization of the nucleus in root-nodules of Leguminosae seems to be preceded by some enlargement. The subject requires further study.

#### CHANGES IN THE CHROMOSOMES.

Following Farmer's statements and similar statements by other English students of malignant animal tumors, the writer has been very much interested to see whether the chromosomes undergo any change in number or location in the rapidly dividing cells of crown-galls and similar plant tumors. The first studies were made on peach tissues but here the nuclei are so small that the determination of the normal number of chromosomes proved difficult. Attempts to get tumors on onion, the normal cytology of which is well known, also failed. The Paris daisy was finally selected. This has large nuclei and the normal number of chromosomes appears to be 16. A study of slides prepared from very young stages of tumors taken from this plant has thus far shown nothing definite except that at least a part of the divisions are mitotic.

The most interesting thing made out in connection with the cell morphology is that first pointed out by Toumey for the almond gall, *viz.*, the occurrence of more than one nucleus in a cell without any evidence of the beginnings of a cell-wall between them. Toumey figures 2, 3, and 4 nuclei in a cell. The writer has seen two well-developed ones in cells of the rose gall.

#### ANTIBODIES.

This is almost a wholly unworked field. The writer has seen nothing corresponding to the self-limited infectious diseases of animals, or which indicates that plants can be preserved by vaccines. The subject is of extreme interest theoretically. Practically it is of less importance, owing to the great number of plants which would have to be inoculated and their slight value individually in comparison either with the labor involved or with the individual value of the higher animals. One attack does not confer immunity on any plant so far as known to the writer. But in this connection it should be understood that we know as yet very little of all that is to be known about this group of plant diseases, and



Fig. 29.\*

\*FIG. 29.—Tumor on sugar-beet produced by a Schizomycete plated from crown-gall of peach. Inoculated Mar. 11, 1908. Photographed May 4, 1908. Nearly natural size. Five plants were inoculated and all contracted the disease. Previously the organism had been passed by the writer twice through peach-trees with production of galls.

it is rather to be expected that some of the many wonderful interrelations now known to exist between the attacking bacterium and the resisting animal body may be found to apply also in a lesser degree to plants.

In 1908 and 1909 the writer and his associates Townsend and Brown obtained some evidence going to show that after Paris daisies have been several times inoculated by *Bact. tumefaciens* with the production of tumors subsequent inoculations by cultures of the same virulence are without effect even on the young tissues of rapidly growing cuttings.

Subsequent experiments showed that at least a part of this supposed increased resistance was due to loss of virulence on the part of the organism. In 1910 my results on these plants were inconclusive, owing to loss of virulence on the part of the cultures used. So

far in 1911 we have not been able to obtain cultures from galls occurring on non-resistant plants, and with virulent cultures from a gall which formed on a resistant plant we have obtained galls as easily on the resistant as on the non-resistant plants, so that the problem is still unsolved.

The writer thought for a time that he had achieved resistance to the olive tubercle on plants freely and repeatedly inoculated, but it may have been only lessened virulence of the organism used. The plants are still under observation. They developed tubercles freely when they were stimulated into more rapid growth and inoculated from a fresh isolation. In fact none of my inoculations on olive have yielded a higher percentage of tubercles, *i. e.*, 105 plants inoculated in 208 places with the formation of 208 groups of tubercles where inoculated, and subsequent

metastasis. The organisms which finally failed on these plants after repeated successful inoculations were old stock cultures of Californian and Genoese origin, *i. e.*, organisms plated several years before from olive tubercles obtained from these localities. The cultures which succeeded so admirably on the same plants under the new conditions, *i. e.*, increased food and water supply, obtained by transplanting from pots to a deep bed, were recent isolations from an olive tubercle collected in Portofino, Italy.

Hiltner maintains that when legumes have been infected with a virulent root-nodule organism one can not thereafter obtain infections on these plants with a less virulent organism, and this appears to be all that has been established for olive tubercle and the crown-galls.

## LITERATURE.

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| <p>1898. SHATTOCK, SAMUEL G. The healing of incisions in vegetable tissues. <i>Journal of Path. and Bact.</i>, Edinburgh and London, 1898, vol. v, pp. 39-58, 2 pl., 6 text figs.</p> <p>1903. HILTNER, L., UND STORMER, K. Neue Untersuchungen über die Wurzelknöllchen der Leguminosen und deren Erreger. <i>Arb. a. d. Biologischen Abt. für Land- und Forstwirtschaft am Kaiser. Gesundheitsamte</i>, 1903, Bd. III, Heft 3, p. 151.</p> | <p>1907. BRÜLLOWA, J. P. Ueber den Selbstschutz der Pflanzenzelle gegen Pilzinfektion. <i>Jahrb. f. Pflz. Krhk.</i>, K. Bot. Garten Petersb., 1907, Nr. 4.</p> <p>1910. ALTEN, H. VON. Zur Thyllenfrage. Callusartige Wucherungen in verletzten Blattstielen von <i>Nuphar luteum</i> Sm. <i>Bot. Ztg.</i>, 1910, Part II, vol. 68, 89-95.</p> |
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\*FIG. 30.—Cross-section of woody part of a young mulberry shoot (3 months old and growing rapidly) showing injuries due to *Bact. mali*, *i. e.*, vessel-walls stained yellowish brown and occupied by tyloses. Bacteria very abundant farther up stem, but comparatively few at this level which is about 35 cm. below the point of inoculation and 30 cm. below any external appearance of disease. The only vessels showing tyloses are those affected by the bacteria. Inoculated near apex of shoot on Feb. 11 1909. Drawn from an unstained free-hand section Mar. 15, 1909. Zeiss 8 mm. obj., and No. 12 ocular

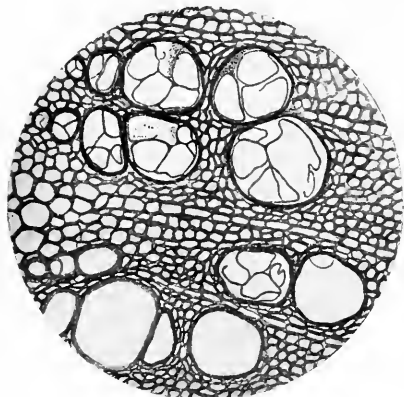


Fig. 30.\*

INDIVIDUAL AND VARIETAL RESISTANCE—WHAT CONSTITUTES IMMUNITY? IMMUNE VARIETIES—INTRA-VARIETAL SELECTION—CROSS-BREEDING FOR RESISTANCE—IS IT POSSIBLE BY SPECIAL FOODS TO OBTAIN RESISTANT PLANTS?

There is a good deal of scattered evidence going to show that resistance to fungous and bacterial diseases differs greatly within the species, *i. e.*, different varieties react variously. The writer has also seen some things which lead him to believe that there is also variation in resistance inside of the variety, particular individuals being more resistant than their fellows.

In pear-blight it is a well-known fact that certain varieties are very sensitive to the disease, for example, Clapp's Favorite and Bartlett blight badly; other varieties are more or less resistant, *e. g.*, Duchess, Kiefer, and Seckel. The same is true of apples: Craig has made lists. It was formerly thought that Le Conte was a blight-proof variety of pear, but this was due to incomplete observation. The Le Conte was grown principally at that time in the southern States where the blight organism was not very widely distributed. Since then it has become distributed in those localities and the Le Conte has suffered severely, whole orchards being destroyed. The Idaho pear also was advertised at one time as blight proof, but many trees of this variety have since been destroyed by blight including the original tree from which the variety was multiplied. The tree was never resistant but, like the Le Conte, in the early years of its cultivation it was not much exposed to the disease. Formerly all pears in California were exempt for the same reason. Now, however, the disease is widespread and destructive. The only evidence of this sort worth very much is that to be obtained in mixed orchards in regions where the disease prevails annually.

Dr. Halsted, in New Jersey, observed that bean-varieties were susceptible in very different degrees to *Bact. phaseoli*. Deane B. Swingle, then of my laboratory, observed the same thing on Arlington Farm, in Virginia. Delacroix has reported the same thing for the bean disease prevalent near Paris. In the matter of potato-rots it has been observed both in Germany and in this country that some varieties are much more liable to attack than others, and when attacked rot worse (see plate 11). In Holland some varieties of hyacinths are known to be much more subject to the yellow disease than others: Consult lists of susceptible and resistant hyacinths under Yellow Disease of Hyacinths. For studies of resistance in varieties of sweet corn, and for a statement respecting variation in susceptibility of sugar-cane to Cobb's disease, see Vol. III.

It has been observed in California in seedling orchards of Persian walnut (*Juglans regia*) that some individuals resist the bacterial walnut blight better than others. The same thing has been observed in the eastern United States in patches of tobacco attacked by the bacterial wilt. The writer has also observed indications of intra-varietal resistance in potato tubers inoculated with *Bacillus phytophthorus*.

We may as well admit that we do not know what constitutes immunity. It is a good subject for study. The factors underlying it are probably partly physical, partly chemical. When they have been brought into clear relief, and this can be done only by the laboratory method, we shall be in a much better position to cope with these destructive diseases, in the presence of many of which we are now so helpless.

Variation inside of the variety suggests that careful selection may in the end lead to the production of many resistant sorts. This has been accomplished already for certain fungous diseases, *e. g.*, cotton-wilt.\*

There are perhaps no absolutely immune races or varieties, but in case of many bacterial diseases there are some which approximate this desirable condition. Generally speaking

\*Orton: U. S. Dept. of Agriculture, Farmers' Bulletin 302 (1907), and Farmers' Bulletin 333 (1908).

these varieties are commercially less desirable than some of the sensitive ones. Frequently they are quite inferior except in hardihood. Systematic cross-breeding experiments are here in order. These should be taken up as a part of the regular work of Departments of Agriculture and Experiment Stations and carried on without interruption for a long series of years—long enough to insure good hybrids of fixed quality. In some cases systematic selection within the limits of susceptible varieties might lead to useful results.

A priori, it would seem that something might be done to increase the resistance of plants to disease by judicious feeding. This subject is also in its infancy. Laurent's experiments point in this direction, but the question is how much can be accepted as really established by him. Jenssen got contradictory results. Also in the writer's experiments, which were made on a large scale two years running, using the green shoots of potatoes, *Bacillus coli* was not induced to take on a parasitic life by any variation in feeding. Similar negative results were obtained with *Bacillus aroideae* and *Bacillus carotovorus*. Moreover, even with a distinct parasite like *Bact. solanacearum*, no conclusive or striking results were obtained. Laurent, however, did not claim that decay could be produced by inoculations from cultures of *B. coli*. The soil in my experiments was Potomac river loam. The fertilizers were lime, sodium nitrate, sulphate of potash, muriate of potash and superphosphate (boneblack). The inoculations were into growing shoots using young agar cultures diffused in sterile water and injected hypodermically. The varieties were Early Rose and Burbank. Three strains of *Bact. solanacearum* were used, i. e., from Maryland, Georgia, and Porto Rico. Each hill of potatoes in a particular plot received at planting time some one of the following doses:

Nitrate of potash.....	8 grams or 16 grams
Sulphate of potash.....	8 grams    16 grams.
Muriate of potash.....	8 grams    16 grams.
Lime.....	25 grams    75 grams.
Boneblack.....	8 grams    16 grams.

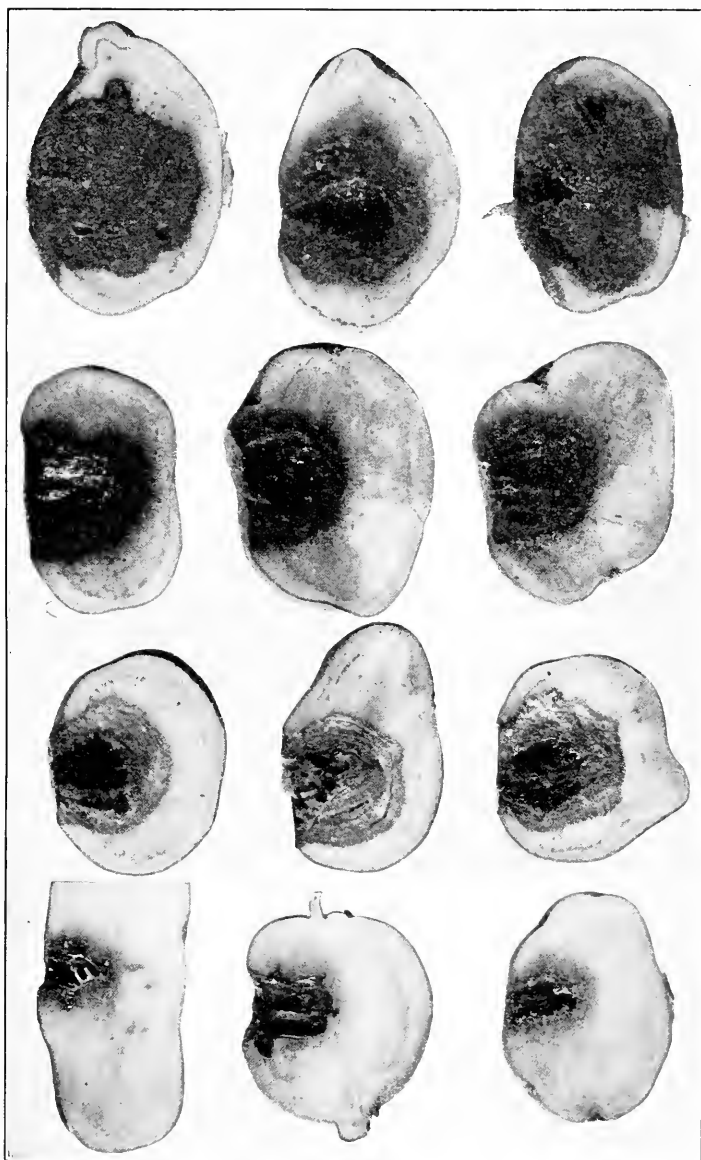
This work should be repeated on other soils, applied to all sorts of diseases and extended in various ways.

Here is a great field for exact experiment. Truckmen, orchardists, and ordinary farmers often stumble on facts of great value, but usually no accurate records are made and consequently the scientific man can not make use of their findings for the benefit of others. My experience has been that it is difficult to persuade a farmer to treat part of a field in some particular way and hold the remainder as a check. He usually wishes to treat it all or not at all. Experienced gardeners have beyond doubt the most exact knowledge on this subject, but theirs also is mostly a rule of thumb which they can not readily impart to others.

## SYMBIOSIS.

Symbiosis according to its Greek derivation is simply *life with* another organism. In biology it has come, however, to have a restricted meaning. We may have *life with* another in which the two organisms are indifferent to each other. This, of course, is not symbiosis. Parasitism also is *life with* another, but the essential idea involved in the concept is antagonism and not what we mean by symbiosis. The latter is a helpful *life with* another, a friendly give and take. The word was invented before we knew much about the conditions to which it was applied. Very few examples of true symbiosis are known, or at least thoroughly worked out. Perhaps there are none in which the giving and taking are equal, and no harm done to either organism. It seems to grade into parasitism. The lichen thallus is an old and familiar example, but here the alga gets the worst of the arrangement, being dwarfed by the fungus, held prisoner and given nothing probably which it could not obtain by itself, unless perhaps in the case of the rock-lichens. Bacterial symbiosis is the only sort falling





### Bacterial Potato Rot.

Four varieties of potatoes inoculated by needle-pricks with a pure culture of *Bacillus phytothorax* (Appel D). Exposed for 8 days at 20° to 25° C. under loosely fitting bell jars to the dry air of the laboratory. Introduced to show rapidity of rot, difference in color—pale in Daisy, dark in Green Mountain; and varying susceptibility—least rot in Gelsamith Thell and most in Factor. Photographed Feb. 1, 1908.



within the scope of this treatise. Is there such a thing? We will run over some of the alleged sorts and let the reader decide for himself. The most discussed case in recent years is the relationship existing between root-nodule bacteria and legumes.

#### ROOT-NODULES OF LEGUMINOSAE.

Should the root-nodules of Leguminosae be cited as examples of symbiosis? The plant submits to distortions and enlargements and final destruction of portions of its roots, giving water, mineral foods, and carbon compounds in exchange for which it receives nitrogen compounds, at least this is the current view. The agricultural chemists appear to be satisfied that the host-plant actually receives the nitrogen and that it is from this source, and is not simply combined nitrogen drawn again to the surface of the earth by the deep feeding roots of legumes, as would be our first thought, considering how readily nitrogen compounds leach out of agricultural soils into the deeper substrata where they can not be reached by surface feeding roots.

The pathologist sees a nodular growth stimulated by the presence of a foreign organism and various phenomena not unlike those of genuine parasitism as Peirce and others have pointed out. We might have, however, a local injury and yet general advantage to the plant if the bacteria really store nitrogen available to the legume.

The organism appears to be able to infect only through very young roots or root-lairs (fig. 21). As soon as the cells of the roots have passed out of a rapidly dividing condition the nodule takes on a definite form and ceases to grow. Subsequently it passes through the same stages of disorganization as other overgrowths in which there is no suggestion of symbiosis. That the micro-organisms infest the interior of the cells, rather than the intercellular spaces, does not alter the case materially. In the end they destroy plasma and nucleus (fig. 31), and the nodule decays. So far then as the morbid anatomy goes we must look upon *Bact. leguminosarum* as a restricted parasite.

Does the host receive something in return? The agriculturist has observed a more luxuriant growth when the nodules are present on the roots of the plants than when they are absent. This, however, by itself might mean only that infections are most abundant on rapidly growing plants, *i. e.*, on plants in a good soil capable of inducing a rapid growth. In recent years, however, in experiments on poor soils, marked increase of growth over that in untreated check plants has been obtained sometimes, by infecting the soil or seeds with this organism at planting time (plate 12). This experiment has yielded the same result, it is said, on a large scale in field practice (see statements by Hiltner and by Moore), and that not once, but many times in widely different localities. On many fields, however, no marked difference has been observed between the check plots and those inoculated with this organism, and sometimes the check plots have given the best returns, even when nodules have been abundant on the roots of the inoculated plants. Sometimes these failures have been on fields already well stocked with nitrogen compounds, but apparently such has not been the case always. Moreover, granting the increased growth associated with an increased number of nodules on the roots it does not necessarily mean free nitrogen assimilated by the bacteria and turned over to the host-plant, unless it can be shown that combined nitrogen is absent from the soil and air, since plants often make an increased growth under the stimulus of weak poisons. This extra

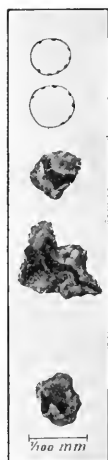


Fig. 31.\*

\*Fig. 31.—Normal and shriveled nuclei from cells in root-nodule of soy-bean. The three lower nuclei are from cells fully occupied by *Bact. leguminosarum*—they are distorted, flattened, have taken the stain deeply, and apparently little is left except skin of nucleus. The two at top of figure are globose and faintly stained except the peripheral chromatin—they are from uninfected cells distributed sparingly among cells destroyed by the bacteria.

growth has, however, been obtained in some instances, it would seem, on soils practically destitute of combined nitrogen, and also, I believe, in an atmosphere destitute of all traces of combined nitrogen, *i. e.*, of ammonia and nitric acid.

Figure 32 shows a pea grown to maturity in a closed space on nitrogen-free sand (on which oats and buckwheat starved) by adding soil extract containing the nitrogen root-nodule organism. This water extract contained only 0.15 mg. of nitrogen, and the hermetically sealed carboy was opened to the date of the photograph (fiftieth day) but three times and then for a few moments only to introduce a measured quantity of pure washed carbon dioxide (6 liters) necessary for the growth of the plants. The capacity of the carboy was

44 liters and while the contents of the air in combined nitrogen was not determined it could not have been over a small fraction of a milligram. The pea grew for a period of 4 months and fruited, yielding a total dry weight of 10.359 grams, of which 233.5 mgs. were nitrogen. Some nitrogen was also recovered from the sand, making (after proper deductions for nitrogen in the seeds, etc.) 248 mgs. of fixed nitrogen. The oats and buckwheat made only a very starved growth and finally perished without fruiting.

It will be remembered that Boussingault got no growth and no nitrogen assimilation in a closed space under sterile conditions. This is exactly Boussingault's experiment plus the addition of soil extract containing root-nodule bacteria, the result being entirely different.

The bacteriologist finds that in pure cultures the root-nodule organism appears to be able to grow on substrata in which there is absence of nitrogen compounds or at least great paucity of such compounds. Mazé states that the organism requires a minimum of combined nitrogen to make a good growth and store nitrogen. He says it can not do so if all initial combined nitrogen is withheld. Others (*c. g.*, Moore) state that it can make a decided growth on

media in which there is no combined nitrogen. According to these observers it makes a good growth on media believed to be free from all nitrogen compounds, and hence the assumption is that it must be able to obtain its nitrogen from the uncombined nitrogen in the air. It is, however, a difficult matter for the ordinary bacteriologist to be assured that all traces of nitrogen compounds are absent from a given culture medium and from the air

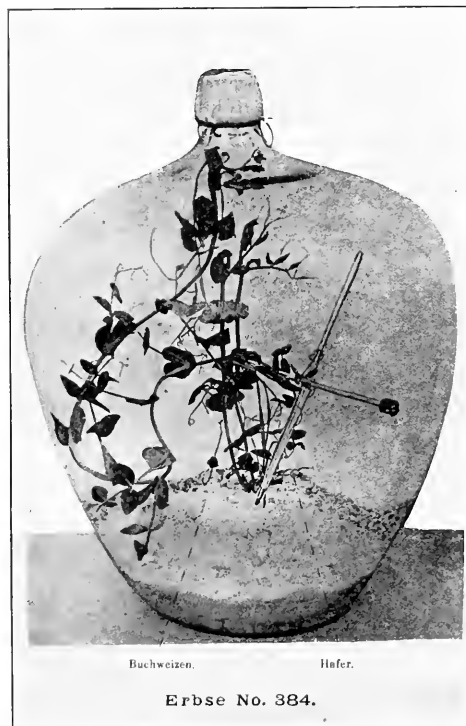


Fig. 32.\*

\*FIG. 32.—Hellriegel and Wilfarth's pea No. 384 grown from germination time in a closed space along with an oat plant and a buckwheat plant on nitrogen-free quartz sand. The sand which had been previously heated to redness was mixed with nitrogen-free nutrient salts and enough twice-distilled water added to approximate 70 per cent of soil saturation. The sand was then inoculated with a little soil extract from earth adapted to peas.

supplied to his flasks. Quite a good many bacteria not known to be assimilators of free nitrogen will make a little growth in some of the so-called nitrogen-free media.

The chemists, therefore, have undertaken to determine whether flask cultures of *Bact. leguminosarum* show any increase of nitrogen as a result of their growth. Most have found no gain, or so slight a gain of nitrogen as to be within the limits of experimental error (see Beyerinck's statement). Mazé is almost the only one who has reported large gains of nitrogen in flask cultures. I do not know what opportunities there are for error in the ordinary nitrogen determinations, but on the bacteriological side I detect a good many suspicious statements in Mazé's papers. Miss Dawson's comment, that Mazé's statements respecting ability of this organism to store nitrogen are to be accepted only with the greatest reserve, appears to be entirely proper. See also Hiltner's comments.

The whole subject of the storage of free nitrogen by this organism in flask cultures and in the plant itself ought to be worked over again carefully by the bacteriologist and chemist. Possibly the root-nodules are only indicators of a fixation of nitrogen which actually takes place in the soil. Certainly it should be determined whether *Bact. leguminosarum* is able to fix nitrogen outside of the plant in agricultural soils both sterilized and unsterilized. The question why the addition of pure cultures of the organism to certain soils does not increase the yield of alfalfa and similar crops, should also be determined. Hiltner has made commendable attempts in this direction. Also, it should be determined why the organism so readily loses its virulence. There are, therefore, several fundamental problems connected with this question of nitrogen fixing in legumes which require further study.

Doubts also exist in some quarters as to whether what is commonly called *Bacillus radicola* has anything whatever to do with the production of the root-nodules. These doubts have been sharply focussed by Gino de Rossi who maintains that a Schizomycete of quite different character is the real cause of the nodules (see abstract), and that we know nothing about its ability to store nitrogen.

Hellriegel and Wilfarth postulated symbiosis. Hiltner seems to waver between symbiosis and parasitism. Mazé maintains that it is not necessary to explain the fixation of atmospheric nitrogen by the hypothesis of symbiosis, the micro-organism being able to gather its own nitrogen without aid from the plant.

#### SYNONYMY OF BACTERIUM LEGUMINOSARUM.

Frank's *Schinzia leguminosarum* appears to be the earliest name and therefore I write *Bacterium leguminosarum* (Frank) as the proper name for the organism causing the root-nodules on *Pisum*, *Vicia*, *Lathyrus*, etc., since it is a Schizomycete, motile by means of a polar flagellum (see vol. I, pp. 165-171). The type form to which this name applies may be taken as that causing the nodules on *Lathyrus* (*Orobis*) *tuberosus*. Should Hiltner's view prevail respecting the existence of two distinct species, Beyerinck's specific name *radicola* may be retained for the organism causing the nodules on *Lupinus*, *Ornithopus*, and soy-bean. The name *Rhizobium beijerinckii* Hiltner and Störmer is inadmissible because there is an earlier *Bacillus beyerinckii* Trevisan, and also because Kirchner's specific name *japonicum*, applied to the organism causing the root-nodules of soy-bean, is earlier. *Bacillus radicola* Beyr. is still earlier and the name *Bacterium radiculum* may be used in place of Hiltner's name. Moreover, there is some doubt whether the name *Rhizobium* should apply at all to the root-nodule organism, since Frank stated his *Rhizobium* to be a micro-coccus. There is no doubt, however, that Frank applied the name *Schinzia leguminosarum* to the zoogloæ strands of this bacterium. That he interpreted them to be fungous filaments does not invalidate the name.

*Pseudorhizobium ramosum* Hartleb (1900) is a name given to a non-infectious organism obtained from root-nodules.

The name *Bacillus beyerinckii* was given by Trevisan (1889) to the white, liquefying, non-pathogenic organism isolated by Beyerinck from root-nodules.

The synonymy of *Bact. leguminosarum* (Frank) is as follows:

- Syn: *Schinzia leguminosarum* Frank (1879);  
*Bacillus radiclecola* Beyerinck (1888);  
*Cladochitrium tuberculum* Vuillemin (1888);  
*Bacterium radiclecola* Prazmowski (1889), Moeller (1892);  
*Phytomyxa leguminosarum* Schröter (1889);  
*Rhizobium leguminosarum* Frank (1890)?;  
*Bacillus ornithopi* Beyerinck (1890);  
*Bacillus fabae* Beyerinck (1890);  
*Rhizobium mutabile*  
*Rh. curvum*  
*Rh. Frankii* var. *majus*  
*Rh. Frankii* var. *minus* } Schneider (1892);  
*Rh. nodosum*  
*Rh. dubium*  
*Rhizobium sphaeroides* Schneider (1894);  
*Micrococcus tuberigenus* Gonnermann (1894);  
*Rhizobium Pasteurianum* Laurent (earlier than 1899 according to Mazé, but the writer has been unable to find the name in any of Laurent's papers).  
*Bacterium* (*Rhizobacterium*) *japonicum* Kirchner (1895) (applied to the soy-bean organism);  
*Rhizobium* *Beijerinckii* Hiltner and Störmer (1903) (applied to organism causing the root-nodules of *Lupinus*, *Ornithopus*, and *Soya*);  
*Rhizobium radiclecola* Hiltner and Störmer (1903) (applied to organism causing the root-nodules of *Pisum*, *Vicia*, *Phaseolus*, etc.);  
*Pseudomonas radiclecola* Moore (1905).

In 1906 Štefan suggested that the root-nodule organism is related to the Myxobacteriaceae.

In 1910, Peklo maintained it to belong with *Actinomyces*.

#### SUMMARY OF LEADING PAPERS.

From the hundreds of pages relating to root-nodules the writer has culled the following statements:

Apparently the first person to discover bacteria in the root-nodules was Woronine (1867). He states that he made his examinations principally upon the common lupin of the gardens (*Lupinus mutabilis* Lindley). He describes the nodule as composed of an interior parenchyma, an exterior parenchyma, and a vascular system between the two, the cells of the interior parenchyma being occupied by enormous numbers of small rods which he figures and describes as bacteria. His exact statement respecting their classification is as follows:

"In all these respects, they have the greatest resemblance to those organisms of doubtful nature which have been designated under the names of *Bacterium* Duj., *Vibrio* Ehrbg., *Zoogloea* Cohn, etc., and we may, without doing violence to the analogies, range them in the same class."

His figures correspond very well to the facts as we now understand them, even to enlargement and shriveling of the nucleus (fig. 33).

The same paper deals with the enlargements on the roots of *Alnus*. In these he says he found a fungus, described as *Schinzia alni*.

Frank (1879) saw the bacterial filaments, sometimes ending in a sharp point in the middle of the cell. To him they were hyphae. The "hyphae" and the small rods and branched bodies were believed to be parts of the same fungus, although Frank was far from certain respecting this. This paper is the one in which he first used the name *Schinzia leguminosarum* (column 397).

Hellriegel and Wilfarth (September 20, 1886) demonstrated the great difference between the nitrogen nutrition of grains and legumes and connected the latter with the presence of the root-nodules.

They made pot experiments, using quartz sand washed many times, nutrient solutions containing all the mineral elements necessary for growth except nitrogen, and watered with pure distilled water (the first part of the distillate being rejected). Under these circumstances they found that the crop of oats or barley was in direct proportion to the amount of nitrate of soda added. When no combined nitrogen was added these grains soon showed nitrogen hunger and uniformly perished.

Under the same conditions, *i.e.*, nitrogen compounds withheld but all other foods added, peas grew remarkably well and produced seeds. Many comparative experiments were made and the results were uniform. It was plain, therefore, that the peas did not obtain their nitrogen from the soil.

Did they obtain it from combined nitrogen present in the air? To settle this question, peas were grown under bell-jars in washed air, *i.e.*, in air from which all the nitric acid and ammonia had been removed, and the growth was just as good as in the unwashed air. Growth was also good on nitrogen-free soil in a closed space in a limited volume of air which could have offered to the plants only a trace of combined nitrogen (fig. 32). The conclusion, therefore, appeared to be irresistible that the peas were in some way able to assimilate free nitrogen.

Boussingault having already shown that legumes can not directly assimilate free nitrogen, the only hypothesis open was some indirect assimilation through the assistance of other organisms. They were led to the conclusion that the root-nodule organism was the factor sought, by having observed that after peas had used up the stored food in the seed there often followed a period of nitrogen-hunger during which growth stopped and the leaves became pale or yellow, but that after a time the green color returned and growth was resumed. In certain plants, however, this resumption of vigorous growth never took place and the roots of such plants were observed to be nearly or quite destitute of root-nodes, whereas the roots of the other plants bore nodules, and the more abundant and better developed these were, the better the growth of the plants appeared to be.

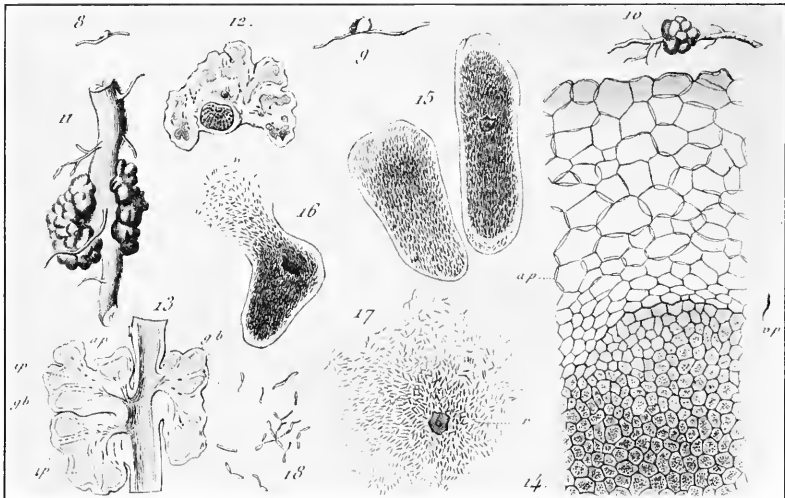


Fig. 33.\*

The next step, therefore, was to add and exclude nodules or nodule products, and so determine the results experimentally. This was done by taking 40 experimental pots containing nitrogen-free soil, holding 30 for checks, and to the other 10 adding 25 cc. of an extract of fertile soil, containing only 1 mg. of nitrogen per pot, all being planted to peas. All passed through a period of nitrogen-hunger, but the plants in the pots inoculated with the soil-extract all regained their green color and grew freely and uniformly. In only 2 of the 30 check pots did the plants grow freely. All the rest continued to show nitrogen-hunger, and some became quite yellow. This difference in color and amount of growth was found to be correlated with the presence or absence of root-nodules.

\*Fig. 33.—Nos. 8 to 11. Stages in development of root-nodules on common garden lupin (*Lupinus mutabilis*).

No. 12. Same in transverse section as seen under a hand-lens.

No. 13. Same as 12 but in longitudinal section; *ap*, external parenchyma; *ip*, internal parenchyma; *gb*, vascular bundles.

No. 14. Cross-section.  $\times 120$ .

No. 15. Mature cells of internal parenchyma containing rod-shaped corpuscles.  $\times 320$ .

No. 16. Cell showing escape of rod-shaped bodies by resorption of membrane.  $\times 320$ .

No. 17. Mass of bacteria surrounding nucleus which has survived cell membrane.  $\times 620$ .

No. 18. Isolated bacteria which have become modified and have ceased to be motile.  $\times 620$ .

After Woronine. Reduced one-eighth.

The effect of the soil-inoculation on legumes differed from the effect of nitrate of soda in that, in the former case after the period of germination, a peculiar and very characteristic hunger-stage supervened which was followed by very energetic and rapid development of the plants.

In two experiments under sterile conditions, the peas grew well in the nitrogen-free sand until the food stored in the seed was exhausted and then dwindled, dying after about 6 leaves had been formed. On these plants not a trace of root-nodules could be found. The same negative result was obtained when the soil-extract was boiled or heated to 70° C. before adding it to the pots.

They concluded, therefore, that the nitrogen assimilation of legumes was in some way connected with root-nodules and the bacteria present therein.

In other words, as expressed in their final report:

There are, therefore, for the Leguminosae two sources of nitrogen, *viz.*, the combined nitrogen of the soil and the elementary nitrogen of the air, the latter being made available to them through the agency of micro-organisms which, to be effective, must enter into a symbiosis with the plant.

Numerous experiments with lupins failed: No successful second growth could be obtained with pea soil and the conclusion was reached that the nodule organism of lupins must be different from that of peas. Only when inoculations were made with soil from a field where lupins grew well did the experimental plants overcome their nitrogen-hunger and do well. On this experiment two check rows of pots were held, one untreated and one inoculated with extract from pea soil. In all three the plants germinated and grew well at first. Then followed a period of starvation, each of the three rows showing equal nitrogen-hunger at the end of a month. Then the first row became green and grew well, while the other two rows continued feeble and red-brown in color. The roots of the first row (inoculated with lupin soil) bore numerous large nodules. The roots of the second row bore none whatever. The roots of the third row (inoculated with pea soil) bore none whatever, except one plant on which a single small nodule was found. *Serradella* behaved like the lupin. Peas, vetches, and beans grew best in the third row.

In Hellriegel's own words:

"Leguminosenknöllchen und Wachstum der Papillionaceen in stickstofffreiem Boden lassen sich willkürlich hervorrufen durch Zusatz von geringen Mengen Kulturboden und Verhindern durch Ausschluss von Mikroorganismen. Bei verschiedenen Papillionaceenarten wirkt nur der Zusatz von gewissen Bodenarten Knöllchen bildend und Wachstum fördernd."

Lawes and Gilbert sum up these experiments very well in the following paragraph:

"The negative result with the *Gramineae*, the negative result with the peas when everything was sterilized, or when the sand was not seeded by the soil-extract, the positive result with the peas when the sand was seeded by the *luminis* soil extract, the negative result with the lupins when their soils were not seeded, or when they were seeded with the same extract as the peas, and the positive result when seeded with the extract from the sandy soil where lupins were growing, seem to exclude any other conclusion than that the micro-organisms supplied by the soil-extracts were essential agents in the process of fixation. Further, the development of nodules on the roots was, to say the least, a coincident of the fixation."

The following year (1887) Dr. Wilfarth stated at the Naturforscher Versammlung in Wiesbaden that they had repeated and extended their experiments with wholly confirmatory results (plate 12). From this time on the scientific world generally accepted their views as may be seen from the following comments of Lawes and Gilbert:

"Thus it may be considered established that the Papillionaceae can take the whole of their nitrogen from the air. \* \* \*

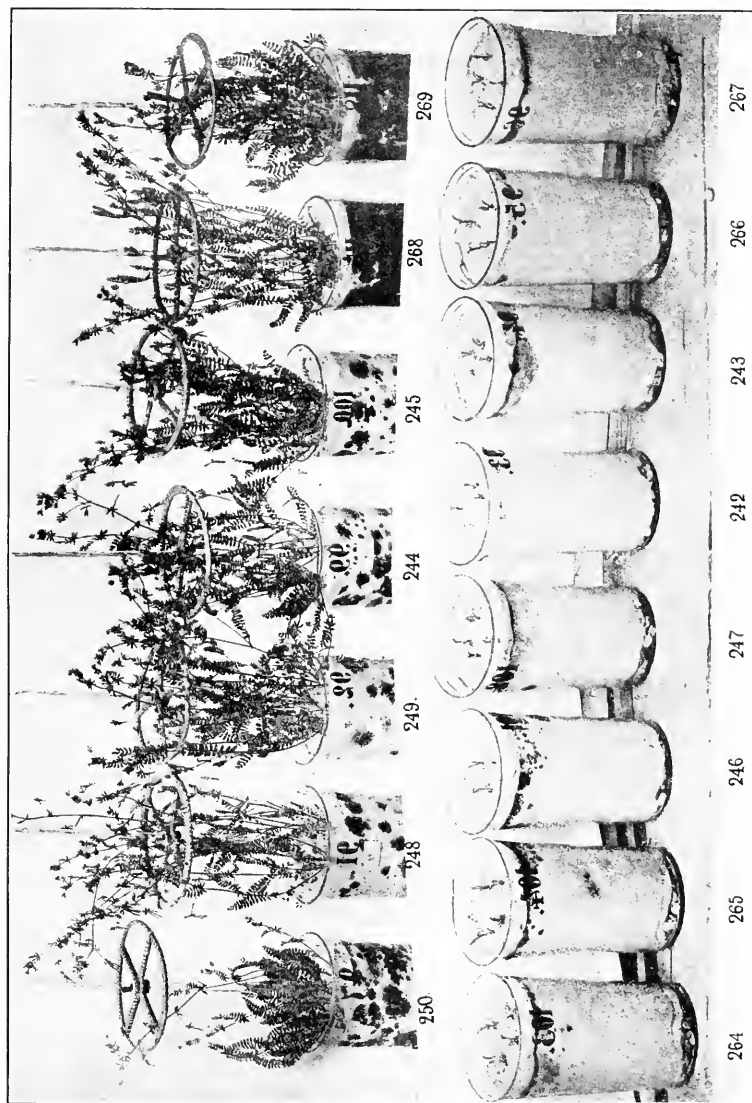
"It will be seen that the results are not only confirmatory of those given by Hellriegel the year before, but that they are even much more definite and striking. Thus, taking no account of the fraction of a milligram of combined nitrogen supplied in the soil-extract, the amount of dry matter produced is nearly 50 times, and the amount of nitrogen assimilated is nearly 100 times, as much with, as without, the soil-extract."

The full account of their experiments was first published in 1888. No figures since published are any more striking or convincing than the six plates which appeared in this epoch-making publication, two of which are here reproduced (plate 12 and fig. 32).

In their experiments with *serradella*, Series C, 1897 (plate 12), each jar contained 4,000 grams of sterilized nitrogen-free quartz sand to which was added the necessary nitrogen-free nutrient salts (monopotassium phosphate, potassium chloride, calcium chloride, and magnesium sulphate). Eight seeds were germinated in each jar, the number of plants being reduced soon after to four. The plants were watered with distilled nitrogen-free water.

To some jars additional fertilizers were added as follows: Nos. 264 and 265 each a small amount of calcium nitrate (41 mgs.); the two end pots of each row (right side) received each 40 grams of calcium carbonate which was sterilized by heat and mixed with the sand previous to planting; No. 250 received some potassium carbonate.





Helfrigel & Willarth's experiments with *Serratella* (*Ornithopus sativa*): Seeds sown May 12, photograph made Aug. 1, plants harvested Oct. 8.

(1) Each jar in the upper row received 25 cc. of water extract from 5 grams of moist soil taken from a sandy field where lupins had grown.  
 (2) The treatment of the jars in the lower row differed as follows: Nos. 264, 265, 246, and 247 received the soil extract *after boiling*; Nos. 242, 243, 266, and 267 received nothing, i. e., no soil extract.



All those plants which had not received nitrates showed nitrogen-hunger early (about time of appearance of third leaf). By June 28, the plants inoculated with the unboiled soil-extract had recovered from their nitrogen-hunger and grew rapidly from this time on. This increased growth of the plants in the upper row was correlated with the presence of nodules on the roots of each one of the plants. There were no root-nodules on the plants in the lower row.

The total grams of dry substance from the upper row (28 plants) was 106.542; the total grams from the lower row (32 plants) was 1.888.

According to Tschirch (1887) the filaments possess no membrane but only a hyaline border layer and, therefore, have nothing in common with fungous hyphae; they are not of a fungous nature. It is very unlikely also that they are plasmodial strands. Tschirch saw and figured the trumpet-like expansions where the filaments penetrate the cell-walls. He believed the membrane was not penetrated. The bacteroids probably are not given off from the filaments. Their variable form is opposed to the view that they are of bacterial origin. It appeared to him rather that the filaments decomposed and then later the bacteroids were developed out of the cell plasma. The filaments and bacteroids are held to be two stages in the differentiation of the cell-contents of the plant itself. The nodules are considered as transitory reserve tissue, especially for albumen; possibly also starch.

Marshall Ward's communication on this subject was read before the Royal Society of London in June 1887, and appears as the last paper in the Philosophical Transactions which was published in 1888, that is at about the same time as Beyerinck's paper in the Botanische Zeitung.

Ward obtained numerous infections in water-cultures by binding on slices of the root-nodules. He saw and figured the entrance of the organisms through the root-hairs in the form of filaments or tubes, as he calls them, and the penetrations of these filaments through the cortex into the tissues where he observed, figured and described the characteristic branching. He also saw the bacteria and bacteroids within the cells. He interpreted the whole phenomenon as one of fungous infection. He regarded the parasite as related to the *Ustilaginaceae* and considered the bacteroids to be yeasts budded from special portions of the hyphae. His description is so full and his figures so distinct that there can be no doubt of his having meant them to apply to this organism. Most of his studies were made on the broad bean, *Vicia faba*.

His cultural work did not lead to any satisfactory results. In his second paper, published in 1890, he says:

"I may here say that these cultures (*i. e.*, as micro-cultures) have given me much trouble, and little results. To obtain pure cultures is a matter of greater difficulty than Beyerinck's paper would lead one to expect, and it is not proposed at present to lay much stress on the evidence got from them."

This second paper figures the entrance of the organism into the root-hairs. First a bright spot appears near the apex of the hair, and from this a little later a filament projects into the interior, and grows toward the cortex. The root-tip curves.

Following Woronine, Beyerinck in Holland was the first man to recognize clearly the nature of the organisms occurring in root-nodules of Leguminosae. These he designated *Bacillus radicola*. His long paper in Bot. Zeit., 1888, called general attention to this subject. The following are some of the statements made in this paper:

The splitting of the primary bark for the emission of the side roots is the special means of entrance of *B. radicola*. The bacteroids stain like *B. radicola* but not intensely. They are of various shapes, branched, round, pear-shaped or bacteria-shaped. They are incapable of growth.

*Melampyrum pratense* has root-nodules containing bacteria (DeVries, Beyerinck). Beyerinck says such nodules also occur on *Rhinanthus major* and on the roots of *Alnus*, *Eleagnus*, and *Myrica*.

While the bacteroids can be found in nearly every cell of the nodule, and occur also in the bark and epidermis of normal roots, the interior of the central cylinder is the special tissue of the bacteroids (see fig. 34).

"The bacteroids are organized albuminoid bodies which the plant has formed out of *Bacillus radicola*, for the purpose of local storage of albumen—therefore an organ of the plant protoplasm, developed from bacteria which have wandered in." (Column 732.)

Two types of nodule are recognized—one in which the bacteria gain the ascendancy and destroy the interior, they themselves remaining alive; the other, in which the nodule, *i. e.*, the host plant, gets the advantage; the bacteria being converted mostly into bacteroids, incapable of growth and furnishing food for the plant. He calls those nodules normal in which no bacteria remain capable of germination, except perhaps in the meristem.

The small threads which pass from cell to cell he considered to be remnants of the nuclear spindle. Sometimes when the bacteria get the upper hand, nucleus and cytoplasm are destroyed.

Beyerinck distinguishes three sorts of bacteroids: (1) normal, (2) a smaller sort called "hem-

mung's bacterioiden," and (3) bladder bacterioids. The third form occurs where the bacteria have multiplied enormously. The *hemmung's bacterioiden* occur outside of the bacterioid tissue in nearly all the outer cells of the nodule, and not rarely in the normal bark of the root.

Beyerinck found that the nodules did not develop on the roots of plants grown in sterilized soil. Frank reached the same conclusion in 1879, Hellriegel and Wilfarth in 1886, and Ward in 1887. Plants in soil rich in humus are sometimes free or nearly free from nodules.

According to Beyerinck there is only one bacterial species, but not all the forms are identical. There are varieties. There is, for instance, a distinct difference between the bacteria occurring in *Vicia*, *Ervum*, *Trifolium*, and *Pisum*, on one hand, and in *Lotus*, *Lupinus*, *Ornithopus*, and *Phascolus* on the other hand. In the large rapidly growing colonies one is most apt to find *B. radicicola* like

ordinary bacteria; in the small slow growing ones there are more branched bacterioids.

He obtained the strongest growing colonies out of the very young nodules, or out of the outer meristematic zone of the older ones in *Vicia faba*, this being the plant he studied most carefully. The inner zone of the meristem yielded more bacterioid elements and slower growing colonies. The same result was obtained with *Lupinus polyphyllus*. This he says is the lupin in which Woronine first saw the bacteria. The nodules of this plant are very large and the swimmers in them are very minute. There are no slime threads and there is no meristem.

The large watery colonies consist of a mixture of rods and swimmers, many motile. The rods exclusive of some long forms are about  $4 \times 1\mu$ . The bacterioids of *Vicia faba* are somewhat larger and average  $5 \times 1\mu$ . The swimmers are very small. Taken from *Vicia faba* they are  $0.9 \times 0.18\mu$ . They are so small that granting them some plasticity they might easily penetrate the pericambium cells without leaving any visible wound. They possess one polar flagellum. This

was inferred from behavior during slow motility rather than actually seen. Motility ceases almost immediately in hydrogen or carbon dioxide. The little slow-growing colonies also contain swimmers.

*B. radicicola* has no special powers of fermentation, oxidation, or reduction. It does not produce spores. It is not harmed by freezing or drying (see fig. 35). Neither diastase nor invertase are produced. Cellulose and starch are not converted. Nitrates are not reduced. Oxygen is liberated from hydrogen peroxide. It is aerobic. It does not liquefy gelatin. Meat-water peptone gelatin is too concentrated for the first cultures (isolations). The addition of 0.25 per cent asparagin is useful in agar cultures. Cohn's solution is too acid for *B. radicicola*. It will not grow in it even after neutralization. Alkaline and neutral solutions are also injurious. For *B. radicicola* from *Trifolium repens* 0.6 per cent  $\frac{N}{4}$  malic acid is useful.

\*FIG. 34.—Planar enlargement of stained section from a small root nodule on soy-bean. Great bulk of section consists of cells much enlarged and occupied by enormous numbers of *Bact. leguminosarum*. Colorless spaces between are occupied by smaller (non-distended) cells free from infection and bearing normal nuclei. Surrounding this central mass is vascular tissue and beyond that cortex (both free from bacteria).

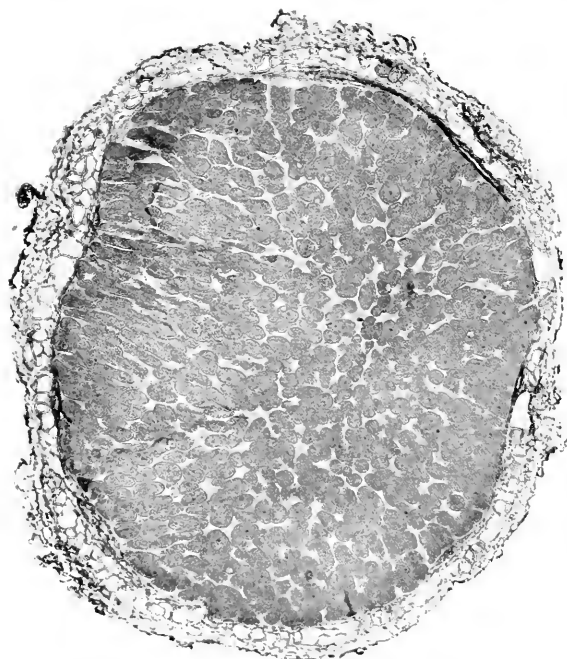


Fig. 34.\*

Beyerinck found swarmers in minute nodules which were still inclosed in the mother root. He divides the root-nodule organisms into groups and varieties as follows:

*Group I.*—This contains the larger more hyaline colonies. Growth absent or difficult on meat peptone gelatin. Growth is favored by cane-sugar or grape-sugar. Swarmers are very minute. The bacteroids are two-armed, globose, or pear-shaped. Meristem is always present in the nodules. The primary bark of the nodule is closed. Slime threads are distinct. The following forms belong here: *B. radicola*, vars. *fabae*, *vicia-hirsutae*, *trifoliorum*, *pisi*, *lathyri*.

*Group II.*—Colonies more cloudy white. Growth better on meat peptone gelatin. Swarmers more rod-shaped, somewhat longer. Bacteroids like the bacteria, that is, seldom branched. Slime threads absent or little developed. Mostly no meristem in the nodules (*Robinia* an exception). Three types occur: (1) *Phascolus* type; (2) *B. radicola*, var. *lupini*; (3) *Robinia* type.

In *Vicia faba*, as the bacteroids are exhausted the color of the cytoplasm changes from reddish to intense green. The bacilli from this plant when grown in *Faba* stem gelatin in a cool place (cellar) were alive and motile at the end of a year. Active cultures can be obtained from all parts of the nodules which have been exhausted by the bacteria. They are present in a living condition therein in great numbers. The result is quite different when the host empties out the contents of the bacteroids. Then it is more and more difficult to get any bacterial growth from the meristem. The longer the bacteria remain in the nodules the more bacteroids occur.

Beyerinck found saprophytes in the nodule tissues mixed in with *B. radicola* and named at least two—*B. luteo albus* and *B. agglomerans*. Another green fluorescent form thought certainly to come from the nodule was identified as *B. fluorescens putidus*. A form resembling *B. radicola* and found in certain nodules was first named *B. radicola liquefaciens*, but subsequently Beyerinck came to regard this as an intruder having nothing to do with their formation. This liquefying organism was afterwards called *Bacterium beyerinckii* by Trevisan.

The bacteroids are found in other parts of the roots than the nodule, but less well developed, e. g., in the root-hairs and epidermis cells. Beyerinck never found them in parts above ground, except once in a stem of *Vicia faba* where inoculated by hypodermic injection.

The bacteroids are always derived from the bacteria. They occur in old cultures as well as in the nodules. The swarmers easily pass through the walls of the Chamberland filter.

When fresh nodules are put into water at room temperature this water clouds first with a mixture of bacteria, of which *B. radicola* is the chief. Later, when the nodules decay, other bacteria appear.

The tissues of legumes have a strong attraction for this organism, as is shown by the fact that in such roots placed in the water any little cracks or wounds are immediately occupied by this organism and the intercellular spaces flooded with it. These roots may be considered as a bacterial trap apparatus.

The infection of the living pericambium of the root must take place through pores, possibly

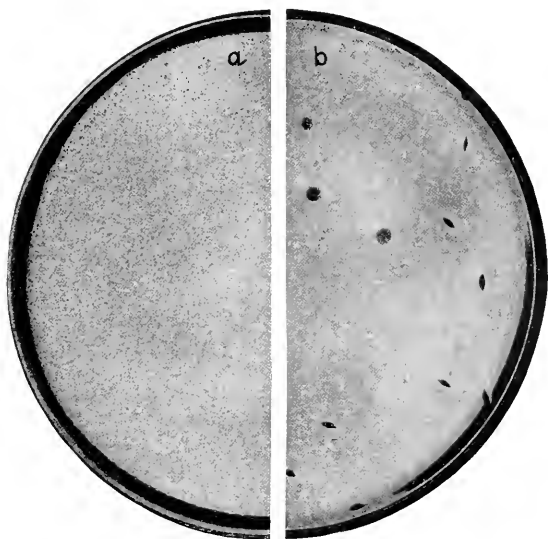


Fig. 35.\*

\*Fig. 35.—Poured-agar plates of *Bact. leguminosarum* from bean, introduced to show effect of repeated freezings: a, Contents of a loop before freezing—several hundred colonies per square centimeter; b, Contents of a similar loop of culture fluid after 7 freezings—less than one colony per each 2 square centimeters. Each freezing lasted half an hour; time between freezing short, i. e., only long enough to thaw out tube in cool water and make necessary plates. Round colonies are on surface; spindle-shaped ones are buried.

those by which the protoplasm of one cell is connected with that of another. The infection is an active and not a passive one.

The nodules are not to be considered as normal organs of the plant. There is no doubt, however, that the plant takes advantage of the presence of the bacteria.

"The papillionaceous tubercles are bacterial galls, useful to the host plant in so far as the normal bacteroids function as providers of albumen—useful to the bacteria in so far as the numerous tubercles filled with bacteria capable of growth function by their decay as centers for the distribution of their occupants."

Beyerinck found no formation of nitrates or nitrites and could not establish the assimilation of free nitrogen in flask cultures. He says that in 14 days the nitrogen increased according to the findings of the chemists but only within the limits of error.

Through its ability to use asparagin and grape-sugar, *B. radicola* has the same nutrition demands as the protoplasm of the host. Beyerinck evidently believed that the *B. radicola* gets its nitrogen from asparagin stored in the plant.

In his Dutch paper published in 1891, Beyerinck states that by additional experiments he succeeded in showing that his *B. radicola* from *Vicia faba* is able to obtain nitrogen from the air. A very great number of active bacteria were used. The increase of nitrogen was, however, so very slight (only about 12 milligrams per 100 cc. on an average in 6 cultures 2 to 3 months old) that he suggests the possibility of its having come from some nitrogen compound present in the air rather than from the free nitrogen. The cultures were kept at 2° to 12° C., higher temperatures being thought to induce loss of function; the weakened cultures take their food more readily from ammonia salts and nitrates than the unweakened ones. By the diffusion method in gelatin he could not be certain of his results, and with the bacillus from the root-nodules of Robinia he could not obtain any increase of nitrogen in 8 weeks.

The cultures were made in Kjeldahl flasks in extract of bean stems (100 gr. germinating sprouts to 1 liter of tap water), with 2 per cent cane-sugar and with one-tenth to one-thirtieth gram of monopotassium phosphate. Sometimes without the latter.

Nitrites in all dilutions are said to be injurious to the growth of the organism. Cane-sugar is a much better source of carbon than asparagin. The earlier statements of Beyerinck respecting the value of asparagin should be rejected as they were obtained from an associated organism confused at that time with *B. radicola*. Peptone is a better source of nitrogen than asparagin, ammonium sulphate, or nitrate of soda or potassa. The growth of *B. radicola* is greatly favored by extract of papillionaceous plants or dilute must extract.

Another point insisted upon is that the concentration of the food stuff should be low, especially the nitrogen compounds and the phosphates. The negative result of his earlier attempts to prove storage of nitrogen is attributed to neglect of this point and to growth at too high temperatures.

In 1897, in his first paper on the subject, Mazé states that he used a bouillon made by heating white beans in water for half an hour at a temperature of 100° C., being careful not to boil it. This bouillon contained about 0.0005 of nitrogen. To it was added 2 per cent saccharose, 1 per cent sodium chloride and traces of bicarbonate of soda. This was solidified by the addition of agar and spread in a thin layer (0 to 4 mm.) on the bottom of large flasks having a side opening by means of which air freed from nitrogen compounds could be introduced into the flasks. Into these tubes he introduced air which had been passed through an asbestos plug, then over copper turnings warmed to just below redness, then through pumice stone saturated with sulphuric acid to remove the free ammonia, and afterwards through pure water. The flasks were set up in series, the last one in connection with the aspirator. This removed 20 liters in 24 hours, not counting the more rapid movement of the atmosphere every morning to remove the gaseous products of respiration accumulated during the latter part of the night. At the end of 15 days the experiment was broken off. Microscopic examination indicated the cultures to be pure and transfers to sterile media also indicated the same thing. An analysis showed that there was a gain in the 15 days of 40.8 mgr. of nitrogen, the initial amount being 62.1 mgr. In a second experiment which lasted also 15 days the gain in nitrogen was 47.5 mgr., as shown by analysis of a mixture of the contents of the two flasks, the initial nitrogen being 70.7 mgr. In a third experiment, using bean bouillon without the agar the experiment was broken off on the sixteenth day, and the results of the analysis of two flasks united showed a gain of 32.4 mgr., the initial amount of nitrogen being 22.4. He concludes that symbiosis is not necessary to explain the fixation of atmospheric nitrogen by the nodule-forming bacteria. This is a property belonging to the organism independent of any influence exercised upon it by the plant. The lack of success experienced by former experimenters he thinks due principally to a defect in the method of experimentation. They placed too little value on the energy necessary to enable the nodule-forming bacilli to convert the nitrogen of the air into an endothermic combination. To place this organism

in a medium deprived of combined nitrogen, obliging it to depend for nourishment from the beginning upon the nitrogen of the atmosphere is to demand of it more than it is able to do.

"We see that the dose of sugar can not fall much below 2 per cent, for the experimenters who have worked with media containing only one per cent of sugar have not found any sensible increase of the nitrogen."

Easy access of air also exercises a very favorable influence on the fixation of nitrogen, and this is easily comprehended, for the rapidity of the combustion of sugar stands in relation to the quantity of oxygen furnished to the cultures. It is because he did not fulfil this condition of aeration that Mr. Beyerinck has observed only a very limited fixation of nitrogen.

Mazé states that the plant must furnish the bacillus 100 grams of starch in order to receive in exchange 1 gram of nitrogen.

"The cultures of the bacillus of the Leguminosae in bean broth, exhaled a strong odor, not without analogy to that which is given off by soft cheeses (brie and camembert)."

According to Mazé's second paper (1898) *Bact. radicola* does not grow in an atmosphere of nitrogen, although it remains alive for some time. Laurent's contradictory results are to be ascribed to a defective experiment, *i. e.*, to traces of oxygen left in his air. The organism is an aërobie. It is greedy of oxygen. It is able to fix free nitrogen without the assistance of the plant.

In fixing nitrogen in flask cultures Mazé states the best result to be when the combined nitrogen was 1 to 200 of the saccharose, the lower limit of the latter being 2 per cent and the upper limit 4 per cent. The minimum limit of combined nitrogen in bouillon cultures is 14 mgr. per 100 cc., and the maximum about 30 mgr. per 100 cc. Mazé's evidence in favor of the storage of nitrogen is increased by another experiment. In three 50 cc. flask cultures there was more than twice as much nitrogen at the end of the experiment as at the beginning, the gain being respectively 12.1 mgr., 12.8 mgr. and 15 mgr. Two other flasks in the same series, differing only slightly in nitrogen and sugar-content, gave no increase of nitrogen and there was only a slight decrease in the amount of sugar. Another experiment is mentioned but here the gains and losses are so slight as to seem within the limits of experimental error (p. 133).

The nitrogen is not all locked up in the organisms; a portion is soluble and will dialyze (about  $\frac{1}{4}$  in a flask culture of 100 cc. diluted to 800 cc. with distilled water, *i. e.*, 8.5 mgr. out of 32.04 mgr.).

In media containing very minute quantities of combined nitrogen the root-nodule organism makes a feeble growth and does not fix free nitrogen. He got no increase of nitrogen in 50 cc. flasks of bean broth containing as little as 3.3 mgr. of combined nitrogen. This agrees with Beyerinck's results, and contradicts Frank's, Prazmowski's, and Laurent's.

Legumin is a good source of nitrogen. Nitrates are better foods than ammonia salts. In ammoniacal bouillon cultures 30 days old there was no increase of nitrogen and very little diminution of the saccharose. The nodule bacteria grew also in sterilized soil free from nitrates, but with no increase of nitrogen (3 months): One experiment only and believed to be insufficient. He states that he did not succeed in isolating from the soil a bacillus capable by itself of producing nodules. Saccharose and dextrose attract these bacteria. Water of germination repels them. They are sensitive to acids. The only chemotactic substances emitted by the roots of legumes are carbohydrates.

Laurent states that the maximum temperature for growth of *Bact. radicola* is 30° C., but Mazé found it grew very well on agar at 35° C., especially after a few transfers.

The branched forms are due to vegetation under harmful conditions as shown by growth in acid media and at 35° C. During the first few transfers at 35° C., and especially at the end of the first 24 hours, they are abundant. In successive transfers as the bacteria become accustomed to this temperature the branched forms disappear entirely. If the branched cultures are diluted with bean bouillon they give rise to unbranched rods. It is impossible to *fix* in the breeder's sense the branched forms by any method of culture. Growth in bean bouillon is prevented by the addition of a small amount of tartaric acid (1:1000).

By sowing very copiously, growth was obtained on slightly alkaline agar to which 1:1000 tartaric or oxalic acid had been added and here pear-shaped forms were found. The pear-shaped and branched forms found in the nodules are ascribed to the injurious action of the acid cell-sap of the host.

The bacteria as isolated from the nodules do not liquefy gelatin. Later Mazé obtained from some of his cultures round forms believed by him to be part of the life cycle, and these liquefied gelatin rapidly.

Mazé states that the round and rod-shaped forms, which he believed at first to be two species, but later forms of one, inoculated separately do not give nodules. Those roots inoculated with mixtures of the two organisms gave numerous nodules. Mazé states that the active rod-shaped form is unable to form nodules. In this he is clearly wrong. He is probably wrong also as to the relationship of the round organism, and this throws more or less doubt on all of his paper. Many of his conclusions

seem to me doubtful. I think he was experimenting with mixed cultures. Especially do I think his theory of alternation of generations, in which *Oospora* is one stage, and an endospore bearing bacillus another stage, not well supported. Possibly, therefore, the nitrogen stored in his flasks may have been due to some other organism than *Bact. leguminosarum*. In no part of his paper are the details of his experiments so stated that one could reproduce them. Apparently he did not make use of poured plates, but depended for isolation on streak-cultures, made in tubes of slant gelatin.

He states that the nodule-producing organism is pathogenic for some species of animals, *e. g.*, rabbits, but this also seems to me not well established by his experiments, since he obtained *Oospora* and an almost round form of very small diameter from the rabbits inoculated with a supposed pure culture of the nodule organism. Abscesses formed, locally, in the inoculated animals.

In 1899, Mazé published a fourth paper on the bacteria of leguminous root-nodules in which he reviews the methods of Salfeld and of Nobbe for inoculating the soil with these bacteria, and in addition gives some of his own experiences.

Concerning Nobbe's work he says:

To justify the method which he recommends, Nobbe starts out with the following hypotheses:

There exist in the soil, neutral forms, capable of forming tubercles on the greater part of the Leguminosae, and forms adapted to definite species. In general the infection of plants takes place by the former, especially in uncultivated soil or in soil which has not borne Leguminosae in a long time. The neutral form is modified profoundly by a passage through a leguminous plant becoming, in this way, incapable of infecting other species.

Bacteria thus adapted constitute a definite race: Thus the species *Bacillus radicola* (Beyerinck) or *Rhizobium pasteurianum* (Laurent) comprises a certain number of races each possessing the ability to infect particular species of Leguminosae. Sometimes a race is able to attach itself to different plants, closely related botanically, but it is not able to utilize atmospheric nitrogen upon these inappropriate hosts.

Mazé then raises the question, not mentioned by Nobbe, as to how these races pass over from one season to another. He says:

"May we conclude that they retain after months and years the ability of their ancestors to live incapable of attaching themselves to plants of other species than the one which previously sheltered them? Nothing would be less justifiable than such an assumption. It has long been known in bacteriology, that all species of bacteria are subject to the influence of the medium on which they live. More than any others, the bacteria of the Leguminosae possess this adaptability which assures the dissemination and preservation of a species."

Mazé claims that forms living in the soil lose, little by little, the characteristics which made them easily identified when taken directly from the nodules. A dilution of soil applied to plants growing in nutrient solutions caused nodules after 15 days. The same dilution inoculated on a series of agar tubes, made for the purpose of obtaining isolated colonies, did not give any forms which corresponded either morphologically or physiologically to the typical bacterium of the nodules. By a long series of passages with all the species obtained from these cultures he states that finally two forms were obtained which he identified by inoculations as the root-nodule organism. From this he draws the conclusion that forms isolated from the soil acquire gradually, when subjected to a medium containing the proper carbohydrate and nitrogen, the ability to elaborate the mucilaginous substance and to fix atmospheric nitrogen. He thinks, therefore, that this ability is very unstable with the bacteria of the Leguminosae. They acquire it in the nodules and lose it in the soil.

He gives the following experiment as proof of this:

He sowed the nodule bacteria on both sterilized and unsterilized soil kept saturated during the whole experiment. On the unsterilized soil, conditions favored the growth of soil bacteria.

At the end of 8 months it was impossible to obtain colonies resembling those which supplied the bacteria sowed. On sterilized soil the bacteria removed from competition with other soil bacteria, retained their initial characteristics after 8 months.

He states also that the characteristics of these races of bacteria at the moment of isolation from the nodules are far from being as distinct as Nobbe claims. Thus, for example, a bacterium coming from one leguminous species is capable of attaching itself to certain other species. Nobbe admits this but thinks that while able to form nodules the bacterium is no longer able on these strange plants to fix nitrogen and so it becomes a parasite which is frequently injurious.

Mazé does not agree with this last statement: Bacteria from any of these plants will fix nitrogen if they have sugar and enough initial nitrogen. The plants all offer that, and he says that the only condition requisite to nitrogen fixation is their ability to penetrate them. This ability, he thinks, depends on the alkalinity or acidity of the soil. He found that lupins inoculated with bacteria from furze and broom formed just as many and as large nodules as those inoculated with bacteria from the lupin, while the checks showed no trace of nodules. The furze and broom came from uncultivated



land which probably had never borne lupins. The only satisfactory explanation which he finds for this is the long adaptation of the bacteria to soil having the same reaction. Those which live in alkaline soils are capable, he thinks, of invading all plants indigenous to such soils while those living in acid (non-calcareous) soils attach themselves indifferently to the lupin, the furze, and the broom.

These facts led him to undertake new experiments. He says that if the reaction of the soil is the essential reason for the existence of two great physiological groups of nodule bacteria it should be sufficient to accustom to acid media a bacterium from alkaline soil, in order to render it capable of producing nodules on the roots of the lupin. This he says he succeeded in demonstrating: Bacteria cultivated 8 months on media of gradually increasing acidity produced nodules on all the lupins inoculated with them. The nodules appeared on the first lateral roots. There were none on the tap roots. Five checks gave only 1 nodule. The same experiments on white lupin gave negative results when grown in mineral solutions, but results were positive on plants normally developed in sand.

In explanation of the influence of the acidity of the soil on the penetration of roots Mazé says:

"If the soil is alkaline, the acidity of the secretions of the roots is neutralized to a certain depth in the tissues. The bacteria, very sensitive to the action of acids, penetrate this layer, attracted by the diffused sugar, but are not able to go farther into the roots."

There must, therefore, he thinks, be forms especially adapted to acid soils.

From his results he concludes that Nobbe's hypothesis is not confirmed either by cultural experiments, or by the physiology of the bacteria. He says that the bacteria which are free in the soil may be grouped according to the reaction of the soil, into two great categories, and that the forms which are found in acid soils are capable of invading only those plants which avoid alkaline soils such as the lupin, furze, and broom.

Concerning the prevalence of the bacteria he says that when they do not manifest themselves by the production of root-nodules it is not because they are absent from the soil but because the conditions for their development are lacking. These conditions are obtained by proper treatment of the soil, and certainly no one would attempt to inoculate with pure cultures a soil that had not been so ameliorated. Hence he thinks that the use of pure cultures does not greatly aid agriculture. This opinion applies to the nodule bacteria of the Leguminosae. It remains to be seen whether the bacteria of alinite are of as little value.

In 1899, Maria Dawson contributed an interesting paper on nitragin and the nodules of leguminous plants. Her investigations were carried on in England in the laboratory of H. Marshall Ward and were suggested by the commercial introduction of nitragin by Nobbe and Hiltner.

Her studies were confined principally to *Vicia hirsuta* and *Pisum sativum*. Each showed palmettely branched nodules within 14 days of sowing.

Various fixatives were tried. The most satisfactory results were obtained by using Flemming's more concentrated solution or absolute alcohol. Hand sections served better than microtome ones for examination of the bacterial filaments within the cells. She found abundant evidence of the parasitic nature of the organism. In fresh material the infection tubes were made visible by treating with Eau de Javelle or potash. In all cases a bright spot of infection was seen either at the tip or at the side of the hair, accompanied by a bladder-like swelling of the hair at the point of attack. Hand sections of fresh material treated as above showed the course of the infection-tube across the cortex and its branching into the deeper cells of the nodule. Trumpet-like swellings where the tubes cross the cell-walls and numerous spherical or pear-shaped swellings on the tube within the cells (previously described by Marshall Ward) were clearly seen, as well as breaks in the tube, each portion ending in a fine point, the points directed toward each other (fig. 36). This also has been seen by others.

The author next attempted to obtain a reagent which would stain the filaments but not the bacteroids. Gold chloride (0.5 per cent) used on fresh material gave some help. Fresh material en masse was left in the stain from 1 to 24 hours for microtome sections, while hand sections were stained for 10 or 15 minutes. In either case the material was quickly washed with water and transferred to a solution of formic acid (0.25 per cent) in the dark for 24 hours, or for the same time to water acidulated with acetic acid, in the light. The sections were then washed well in water and placed in formic glycerin, or if intended for imbedding, the material was transferred gradually to absolute alcohol and thence to paraffin. By this method the contents of the filament stained deeply and had the vague appearance of being made up of numerous short rodlets. The limiting layer remained colorless.

This hint as to the nature of the filament was successfully supplemented by the use of Strasburger's method for differentiating fungous hyphae in the tissues of the host. She found the best method of treatment to be as follows: Sections hardened in alcohol (best without previous treatment with chromic or osmic acid) are placed for about two hours in alcoholic potash (one part 5 per cent potash to three parts absolute alcohol) and then passed into Eau de Javelle for 10 minutes. From

this solution they are transferred to the dye which is prepared by mixing an alcoholic solution of aniline blue with orseillin, drop by drop, until a violet solution is obtained. The mixture is acidulated with a few drops of glacial acetic acid. The sections remain in the stain for two hours and are then transferred to dilute glycerin and finally mounted in glycerin. By this method the rodlets were plainly differentiated.

Where swellings on the filaments occur these rodlets are very numerous and finally the tube bursts and the rodlets are liberated into the cell-cavity. The bursting of the filaments, or tubes as Miss Dawson calls them, is a normal phenomenon. A transverse section of the nodule showed a filament crossing the cell-wall, the figure given resembles an ordinary sieve-plate, but the relation of the bacteria to the plate is rather obscure. She thinks the rodlets actually pierce the wall, absorbing only the middle lamella. Further confirmation of the general results was obtained by staining with methyl violet and fuchsin, though the former method was the more successful.

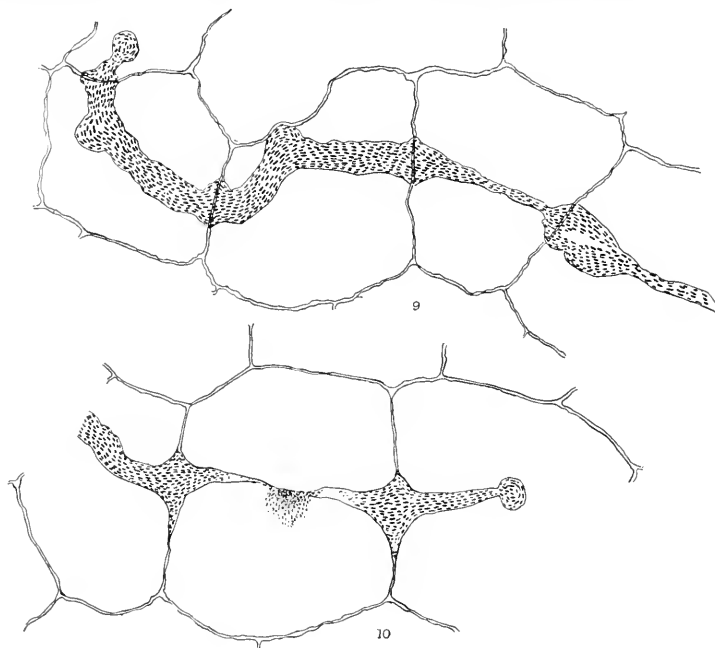


Fig. 36.\*

In some cases the filaments were in close contact with the nucleus but she did not find this relation constant. She says:

"In sections of older tubercles the thicker filaments crossing the cortex are no longer to be seen but those in the main tissue of the tubercles persist until decay has set in."

She says further:

"The tube, therefore, is actually formed by the parasite as it grows down the hair, and does not arise from the plasma of the host plant."

A variety of opinions exist as to the presence and constitution of a membrane bounding the filaments. This author maintains that her results confirm Marshall Ward's claim that a membrane is present, but she failed to detect in it cellulose or chitin. The presence of mucilage she considers doubtful. She used Wisselingh's method for the detection of chitin. This is as follows: Sections of alcoholic material are heated in concentrated potash to 160° C. for two hours. After cooling they are

\*FIG. 36. Longitudinal section (9) of root-nodule of *Pisum sativum* stained with aniline blue and orseillin, showing rodlets of *Bact. leguminosarum* within filaments. Longitudinal section (10) of root-nodule of *Pisum sativum* stained with methyl violet and fuchsin, showing liberation of rodlets from filaments. After Maria Dawson.

washed in 90 per cent alcohol and then stained with iodine and sulphuric acid. If chitin be present a beautiful pink stain is given to the hyphae while the cells of the host take on the usual blue color of cellulose.

Staining the infection tube within the root-hair shows it to consist of a chain of rodlets like those found in the filaments within the nodule. The growing point of these filaments is, as Frank asserted, a diffuse open end. In fresh material this open end generally shows a rosette of refringent granules, suggesting the exudation of a ferment by the contained organism.

In plants growing in ordinary soil only one infection tube was found entering each nodule, while among those experimentally infected, several tubes from as many root-hairs often entered the same nodule.

The bacteroids of all species examined were the same in character, consisting of small straight X-shaped or Y-shaped rodlets which stain very readily. At the close of the vegetative period the older nodules are empty sac-like bodies, devoid of bacteroids, but containing a few straight rodlets and some proteid bodies. This observation she thinks supports the theory that the bacteroids have been absorbed by the plant along with any nitrogen contained in them.

The characters thus far determined are opposed to the view that this organism is one of the higher fungi.

The mean size of the rodlets is given as  $0.99 \times 3.3\mu$ . Experiments to determine the life history of the rodlets were undertaken by Miss Dawson. She secured pure cultures and by dilution isolated them in drop cultures for continuous microscopic investigation. To secure pure cultures large nodules were washed with mercuric chloride and alcohol, then with distilled water and cut across with a sharp razor. Streak cultures were then made on slant tubes of gelatin with a sterile platinum needle which had pierced the cut surface. From such streaked cultures unmixed cultures were obtained by a series of plates, and slant tubes were then infected for future use. The multiplication of the rodlets by division (2 to 4 hours) was successfully followed in hanging drops, but in no case was the formation of bacteroids seen. The organism is aerobic.

An attempt was made to grow the organism on dead roots. Pea seeds were germinated between layers of cotton wool till the radicles were an inch long, then dropped into sterile tubes containing wet plugs of cotton and steamed in a water bath for ten minutes. After cooling the roots were infected with nitragin and kept in the dark. In 10 days good growth was obtained, seemingly of the organism sought, but attempts to get pure cultures failed because of the presence of liquefying bacteria.

Tests were then made of the ability of nitragin to produce root-nodules. Inoculations were made according to directions, both by rubbing the seeds with the nitragin rubbed up in water and by pouring such water over the soil where the seeds were to be planted. Sterile water and utensils were used. No attempt to sterilize seeds before sowing was made and check experiments, she says, justified this, showing that the Leguminosae are not hereditarily infected with the nodule organism.

Results from inoculations were in all cases positive. In 4 out of 6 experiments the controls remained free from nodules, while in one of the inoculated sets the entire 20 plants developed nodules. The nitragin from *Pisum* and *Vicia* was apparently identical in action and the latter when applied to seeds of *Lathyrus aphaca* produced a considerable increase in positive results in comparison with untreated plants. A similar increase resulted from the use of the nitragin supplied for *Onobrychis* and *Lupinus* upon seeds of *Vicia hirsuta*. The appearance of nodules on controls illustrates, she says, the difficulty of keeping soil or sand free for many weeks from this ubiquitous organism. [In all cases an attempt at least should have been made to sterilize the surface of the seeds. If controls become affected how then are we certain what caused the effects produced in the inoculated plants?]

Miss Dawson agrees with Zinsser that the bacteroids do not occur in the aerial organs of the plant or elsewhere in it, except in the nodules. Zinsser attempted direct infection of the roots under conditions which could be observed, that is, by injecting the organism into the tissues and by stroking the rootlets with needles dipped in the inoculating material. His results were in both cases negative.

Since infections in nature occur always through the root-hairs Miss Dawson used external applications only. Seedlings whose roots were infected by drops of water containing nitragin or were dipped entirely into the solution grew vigorously but gave negative results. This suggested that either the organism must pass through the soil, or that infection is impossible after the root has grown beyond a certain stage. Further experiments showed that the second hypothesis is the correct one, since placing the bacteria on the radicle shortly after germination gave very positive results, in one case fully 27 root-hairs side by side showing infection tubes. In all cases infection resulted within 12 days of inoculation.

The question is undecided as to conditions regulating the entrance of these organisms, since full-grown hairs often show tubes just beginning growth, while infection of the root-hairs is perfectly easy and certain if the organism is placed on roots that have not yet formed hairs. She thinks that the

infection often takes place in the root-hair just as it is emerging from the root. In one case she observed several very small root-hairs, scarcely larger than the cell from which they arose, penetrated by infection tubes which had already reached and entered the outer layer of the root-cells.

Further experiments were made to determine the possible inhibiting effect of carbon dioxide collecting about the roots when seedlings were grown in tubes: These were negative. Caustic potash was introduced into some of the tubes to absorb the carbon dioxide. Plants flourished equally well with potash and without potash, the only positive result occurring on a seedling in a tube without potash. To test the effect produced by changing conditions under which plants were growing, seedlings grown one week in sterilized sand with nutrient salts were removed, infected with nitragin and fixed in tubes. Others were germinated in the same manner, but returned to fresh sand after inoculation. Others were germinated in tubes, and when the roots were two inches long were inoculated and again fixed in tubes. Three weeks after inoculation the most positive results were where conditions had remained as far as possible unchanged.

Examination of the nitragin and of fresh subcultures therefrom showed it to "consist of immense numbers of very minute bodies, scarcely longer than broad, all non-motile and similar in size and shape. No trace is found of the variety of shapes exhibited by the bacteroids."

In 1900, Maria Dawson published "Further Investigations on the Nature and Functions of the Nodules of Leguminous Plants," from which I abstract as follows:

*Phaseolus* shows no nodules for at least 3 weeks after germination, and these are confined almost entirely to small lateral roots. Large nodules contain a considerable quantity of starch. Situated from one to three cells below the surface of the nodule is a layer containing large crystals of calcium oxalate. A similar layer was found in *Desmodium*. Bacterial filaments strictly comparable to those in *Pisum*, were found in small nodules, never in those larger than a pin's head, and only once was an infection tube seen within a root-hair. In this genus root-hairs are few in number.

These results suggest that in *Phaseolus* the germs in the absence of root-hairs can enter the host directly across the piliferous layer, and that within the root they can continue their growth for a while with or without the formation of a filamentous structure.

*Acacia* agrees with *Phaseolus* in having filaments in very young nodules but not in the older. In this as well as in *Phaseolus* it is possible that we have an intermediate stage in the adaptation of the parasite.

A detailed study was made of the nodules of *Desmodium gyrans* and pure cultures were made of the organism concerned in their formation, since this was of an unusually large size ( $1.3 \times 3$  to  $7 \mu$ ). Similar large forms occur in *Acacia*, *Flemingia*, *Carmichaelia*, *Coronilla*, and *Psoralea*. In section, the nodules of *Desmodium* resemble those of *Lupinus* and *Phaseolus*, but a new feature was noted. This was the presence in material hardened in absolute alcohol of bright, apple-green bodies, one, as a rule, in each cell. The nature of these bodies has not as yet been determined. They are promptly and completely soluble in 5 per cent potash. These bodies occur also in the nodule-cells of *Robinia pseudacacia* and in both cases digestion in gastric juice caused the green color to become more conspicuous. Gastric juice was also found useful for rendering the bacterial filaments conspicuous in sections of *Pisum*, *Vicia*, and *Robinia*.

Upon *Cassia* roots she did not observe the formation of nodules. The older roots of this genus are jet black, contrasting strongly with the pale, greenish-yellow root tips.

The author discusses further the biology of the bacteroids. The time required for the growth of a lateral branch in hanging drop cultures averaged about 1.5 to 2 hours. She isolated the nodule organism by a series of separations on tube and plate cultures, and from pure cultures on gelatin microscopic preparations were made. A triple series of these cultures was kept under observation and referred to in her descriptions as A, B, and C. They were:

- (A) Organisms from sub-cultures of commercial nitragin for *Pisum sativum*.
- (B) Organisms cultivated directly from the nodules of *Pisum sativum*.
- (C) Organisms cultivated from the nodules of *Desmodium gyrans*.

Gelatin plates made from nitragin yielded what appeared to be pure cultures, the colonies looking alike. Further studies led the author to consider the "Nitragin" examined by her as a bacteriologically pure culture. She says:

"The general characters of the three organisms are alike, though small differences are noticeable in aggregate cultures. They all grow readily on gelatin, or agar, containing a decoction of pea stems and leaves, asparagin, and a small percentage of sugar, and giving a very faintly acid reaction."

On broth-agar no growth occurred at  $20^{\circ}$ , or at  $25^{\circ}$  to  $30^{\circ}$  C. On broth-gelatin at  $20^{\circ}$  C. growth was extremely slow. Beyerinck also states that his *Bacillus radicola* grows very slowly in meat-juice peptone gelatin. No change occurred in milk kept for 3 weeks at  $15^{\circ}$  C. either in consistency or litmus reaction.

"For the purpose of a close comparison of 'nitragin' with organisms direct from the nodule, grown on gelatin, a double series of tubes (gelatin 10 per cent; asparagin 0.25 per cent; saccharose 1 per cent; pea extract) were infected with cultures A and B respectively and kept under the same conditions at ordinary temperatures. From these at intervals of 24 hours, preparations were made, and stained with carbol fuchsin."

The nodule bacteria grow most rapidly on gelatin at 15° to 18° C. and on agar at 30° to 35° C.

The microscopic characters of the organisms in both cases were quite similar, but those of A (nitragin), after 24 hours' growth, had enlarged to nearly twice their former size. After 48 hours' growth, the maximum size was reached with a few X and Y forms present. The size gradually diminished until after 5 days the original size was reached. No X and Y forms were seen in the last preparations of either type.

In drop cultures of the organism from *Desmodium*, colonies 8 to 10 days old consisted of numerous small rodlets, with some long rods and intermediate stages.

In all three types, the formation of a typical colony was observed in a hanging drop of 5 or 2.5 per cent gelatin. Within 5 days the colony reached its maximum size (28μ in diameter). From this time it slowly disintegrated when it was obvious that many X and Y forms were present, the latter predominating. Some rods were curved, others straight. Several individuals were in turn observed for the formation of bacteroids. The fact that X and Y forms arise as a distinct branching of the rods was repeatedly demonstrated (fig. 37). In 14 days the branched forms had disappeared from

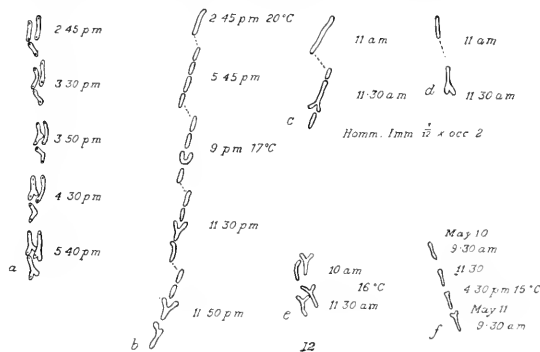


Fig. 37.\*

the drop under observation. This now contained a few large rods and large numbers of small ones with intermediate stages.

"When branching is about to take place, the rod, as a rule, becomes at first curved, and then from the point of greatest curvature on the convex side a lateral branch grows out, giving the resultant Y form. In other cases both arms, here usually very short ones, appear to form simultaneously, suggesting a kind of dichotomous branching of the already swollen head of the rod."

She also says that in pure cultures on nutrient gelatin large numbers of X and Y forms occurred, suggesting a kind of dichotomous branching of the already swollen head of the rod.

The bacteria stain by Gram. On treating with methylene blue or carbol fuchsin deeply stained bodies appeared at the ends of the cell and also one or more in other parts.

In the branched forms they are seen at the extremities of the arms, at the angles, and sometimes along the course of the main rod. These are doubtless the bodies which have been described as spores by Schneider, and as cocci by Frank.

She says further: "The daughter cells produced are approximately equal, but there does not appear to be absolute regularity in the sequence of further divisions, most frequently both halves divide again in a regular manner; sometimes, however, only one divides, whilst the other undergoes no further change or takes on the 'bacteroid' form. Again, both daughter rods resulting from a division may become thus transformed, though rarely simultaneously, into bacteroids."

These observations support the theory of Beyerinck (1888) whose conclusions respecting branching were drawn not from the continuous study of single rods but from observations upon numerous individuals assumed to represent different stages in the formation of bacteroids.

\*FIG. 37.—Stages in formation of branched bacteroids in pure cultures from root-nodules of *Desmodium gyrans*, observed in hanging drops of nutrient gelatin. After Maria Dawson.

In some cases motile stages appeared in cultures a week or more old. These never traveled far; often one end remained stationary while the other oscillated to and fro.

In plate-cultures of organisms obtained from nodules of different genera of Leguminosae no radical differences were noticed. Tiny cream-like colonies become visible after two days' cultivation on nutrient gelatin.

"After four days a distinction [under the microscope] is obvious between the flat, pale yellowish-brown emerged colonies and the submerged ones which show a manubrium either central or on the margin, and are darker in color and more opaque than the former. The submerged colonies frequently become confluent. No liquefaction of the gelatin had taken place after two months."

On gelatin the surface colonies of the organism from nodules of *Desmodium gyrans* become distinctly domed when the size of a pin's head.

"In streak cultures slight differences in the mode of growth of the various types occur, but no corresponding microscopic differences could be seen."

In stab cultures no liquefaction of the gelatin occurred. As in streak cultures, the surface growth in A was at first more pearly than in B and C.

Regarding the systematic position of this organism as affected by her investigations, Miss Dawson says:

It may now be assumed as proved beyond question that we are dealing with independent organisms which have become very specially adapted for life within the cells of leguminous plants—a specialization which varies apparently with different hosts. \* \* \* It is impossible, as yet, to assign them to any other group, if not to the schizomycetes.

She also says:

"On the other hand, if branched forms are to be included among true bacteria, in accordance with the views of the aforementioned investigators, the organisms (which it is convenient to continue to describe as Rhizobium) could certainly claim to be classified with them, and in this case the branched individuals would probably be regarded as involution forms. As, however, such a classification would involve a new definition of bacteria, it seems advisable to adhere to the view that the organisms are in reality either very primitive or very degenerate fungi."\*

A further conclusion based on the experiments described in this paper, in addition to others not recorded, is that there is only one organism capable of forming nodules upon the roots of leguminous plants.

In 1900, Hiltner published a long paper on the causes which affect the size, number, location and activity of the root-nodules of the Leguminosae.

He takes as a motto the statement of Beyerinck that a very subtle balance must exist between the growth of the two organisms, the bacterium and the leguminous plant. This, he thinks, is not a purely symbiotic relation, but a contest, in which a greater part of the penetrating bacteria are destroyed, and in which the host plant also suffers evident injury, but a relation which, nevertheless, is advantageous to both in the end. His experience covers a period of 10 years. The following is an abstract of this paper.

Prazmowski, he says, was the first to demonstrate the true method of infection through the root-hairs. He showed in the pea that only young hairs near the root-tip are penetrated by the bacteria. The attacked hairs curl at the tip, and in their interior there soon appears a shining button-shaped body from which a filament filled with bacteria grows along the root-hair and penetrates into the root, boring through cell-membranes. Some of Hiltner's own experiments on the subject were as follows:

He carefully washed and sterilized with absolute alcohol and corrosive sublimate, mature pea nodules, and rubbed them up with a little water. The whole mass was then filtered repeatedly through a Chamberland filter. The result was a clear liquid free as a rule from bacteria but containing soluble secretions from the bacteroids. Young pea plants, growing in nitrogen-free nutrient solutions were inoculated with a small amount of this filtrate. After a short time numerous young root-hairs showed the same phenomena of curling as did hairs attacked by bacteria. As no nodules were formed, however, it was evident the bacteria had been retained by the filter.

When seedlings of *Lathyrus sylvestris* were inoculated with the same filtrate no curling of the root-hairs occurred for a long time, i. e., not until after the supply of nitrates in the seed had been exhausted. Then when the plants began to hunger the hairs on the youngest roots showed the curling. Pea seedlings inoculated with a filtrate from *Lathyrus* nodules gave like results. These experiments indicate that excretions from the nodule bacteria of one species are able to influence only weakened plants of other species.

\*In opposition to this view it may be stated that branched forms are not peculiar to this organism but common to many bacteria when growing under adverse conditions e. g., in the presence of acids, and these are to be regarded as degeneration forms. Such forms are either dead, or if capable of further growth return to the original form when placed on suitable media.

The membrane of the root-hair is not completely dissolved by this process, but becomes swollen and thus penetrable by the bacteria.

The above results lead to the conclusion that the nodule bacteria of different species do not entirely agree, at least in their physiological peculiarities, and hence to the question whether they comprise one or more species.

Hiltner obtained nodules on the roots of the East Indian *Acacia lophanta*, grown in sterilized nitrogen-free sand, by inoculating them with bacteria from peas and locust. This shows that at least not every genus of Leguminosae requires a special species of bacteria for the formation of nodules. On the other hand, Kirchner observed that, among about 100 species of Leguminosae grown yearly in the garden at Hohenheim, only the soy-beans were free from nodules. When these were inoculated with earth from Japan in which soy-beans had grown, large active nodules appeared. Hiltner at Tharandt in experiments covering several years also found *Soja hispida* always nodule free. Although morphologically the bacteria of these nodules could not be distinguished from native nodule bacteria, Kirchner considered the physiological and biological differences sufficient to warrant their recognition as a new species, *Bacterium (Rhizobacterium) japonicum*. This conclusion is not shared by Cohn or Naudin, who found nodules on soy-beans which had not been inoculated.

Hiltner states that in his experiments in recent years no nodules were formed on his uninoculated plants, except in some of the water cultures which are extremely difficult to keep pure, and that hence, when inoculations produced nodules, the bacteria used must have been the nodule-forming bacteria.

He found the bacteria of the most various Leguminosae, even of the Mimosae and Caesalpinae, extraordinarily alike morphologically. He could find no constant difference, nor could he regularly demonstrate the two groups of Beyerinck, much less the differences described by Gonnermann. On the other hand he thinks that the artificial media generally used for the culture of nodule bacteria, and on which bacteria from peas, clover, vetch, and *Robinia* flourish, is extremely unsuitable for serradella and lupin bacteria. Such medium consists of gelatin weakly acidified with malic acid, and containing an extract of Leguminosae and asparagin and grape-sugar. Concerning other differences Hiltner says:

"The investigations at Tharandt, however, have plainly shown a great difference, both biological and physiological, in the bacteria isolated from nodules of different leguminous plants. It was evident, from the most carefully conducted inoculation experiments that the bacteria from nodules of a definite species of leguminous plant, were most active upon this same species, both as regards rapidity in the formation of nodules, and their nitrogen-gathering activity. On other species of the same genus, or of the same group, however, they generally showed a materially decreased activity, and finally, as a rule, such activity disappeared completely in genera which were farther removed systematically."

Within the Trifoliaceae a mutual transfer of bacteria was less successful than between *Pisum* and *Vicia*. Very active bacteria from *Trifolium pratense* failed to produce nodules on *Medicago sativa* grown in a sterilized mixture of sand and earth.

The lupins showed to a marked degree this difference between species of the same genus. Even the bacteria from *Lupinus luteus* and *L. angustifolius* behaved very differently and Kirchner found among 14 species of lupin grown for several years in close proximity, and even mingled, that while 12 formed nodules from the start, *Lupinus hirsutus* and *L. subcarneus* were completely free from them. For the most part *Robinia pseudacacia* appeared to be very exclusive. It required much time to produce nodules even when bacteria from most closely related genera, e. g., *Caragana*, were employed.

In contrast to *Robinia* the genus *Phaseolus* was easily infected by bacteria from the nodules of other legumes. When inoculated with bacteria from *Pisum* and *Robinia* it produced nodules, which, however, were inactive or nearly so as regards nitrogen fixation. Bacteria, from *Phaseolus* on the other hand, formed weakly active nodules on *Pisum*, but had no effect on *Robinia*.

All these results lead to the probable conclusion that the different nodule-bacteria of the Leguminosae are really only adaptation forms of the same species. Laurent's experiments in which he obtained nodules on peas by needle-prick inoculations with bacteria from 30 different (unnamed) species of Leguminosae, seems to support this conclusion, although he had no check plants, and Zinsser's experiments are contrary.

A study of the bacterioids from the nodules of different species gave the same result. Although sometimes very different in form, the transformations which took place permitted the conclusion that the nodule bacteria of the Leguminosae represent a single, extraordinarily polymorphic species, a conclusion which agrees with Beyerinck's observations on the bacterioids in an old culture from *Phaseolus*.

Hiltner states also that he and Nobbe succeeded in transforming pea-bacteria into forms identical with bean-bacteria in their activity on peas and beans, as well as in the form of their bacterioids.

This they did by means of repeated inoculations on bean plants. The first infection produced inactive nodules. Bacteria from these nodules, kept during the winter on gelatin containing an extract of leguminous plants, when inoculated on bean plants produced comparatively active nodules, while the nodules formed by them on peas were less active than those due to normal pea-bacteria. The following table gives the results for two average plants in each experiment:

1. Uninoculated bean plants.....	0 nodules.
2. Bean plants inoculated with normal pea-bacteria.....	255
3. Bean plants inoculated with pea-bacteria from bean nodules.....	446
4. Bean plants inoculated with bean-bacteria.....	575

From this it is plain that the three cultures of bacteria possess very different degrees of virulence for the bean.

Hiltner says that he is almost completely convinced that the various nodule bacteria are only adaptation forms, a conclusion which is likewise reached by Mazé, who recognized two groups, differing in the reaction of the soil in which they are found, capable of infecting only plants native to soil of the same reaction, but capable also of being transformed by gradual alteration of their nutrient substratum. However, the failure to obtain cross-inoculations from beans to locusts, both native on calcareous soils, does not bear out Mazé's statement that nodule bacteria from calcareous soils can infect all lime-loving plants.

According to Hiltner, the amount of inoculating material used does not influence number, size, or activity of nodules. He used an emulsion of distilled water and bacteria from pure cultures, in one case 100 times stronger, in the other case 100 times weaker than his normal mixture. The plants tried were vetches. The results were equal, except that in the case of large doses the roots remained smaller, probably because the plant had to exert more energy to keep the root-nodule formation normal.

When a given amount of inoculating material was applied (*a*) at once (*b*) in 3 doses, no constant difference in nodule formation was observed. If there were fewer nodules on a particular plant they were individually larger. In all cases the amount of nodule formation bore a definite and constant relation to the aerial parts of the plant.

The varying degrees of virulence, and hence the differences in the effects produced by the same bacterium, were not formerly understood. Thus nitrogen often failed to produce results because the bacteria used were from nodules produced by weakly virulent forms. At other times it was eminently successful because virulent bacteria chanced to cause infection in the nodules used in its preparation. To make nitrogen really successful, cultures should be made from the nodules of plants grown in soil which has repeatedly borne the same species, and whose bacteria have, in consequence, lived repeatedly in the nodules of that species.

Although the balance between plant and bacteria is not disturbed by the amount of inoculating material used, it is altered by the quality of such material. Active nodules make the plant immune against bacteria of the same or of lower virulence than the ones which formed the nodules. Only bacteria of higher virulence are able to penetrate the roots. This fact is said to stand alone in the plant world.

Hiltner observed that *Robinia* plants like other Leguminosae, produced only small, inactive nodules when grown in nutrient solutions which covered the roots completely. When, however, some of the solution was poured off a strikingly rapid growth took place in the nodules on the parts of the roots above the solution, increasing the growth of the plant in a marked degree, while the submerged part remained free from nodules, even when repeatedly inoculated with pure cultures and with the contents of nodules. His two figures are very striking.

This result, which supports the theory of immunity is also in agreement with the observed fact that, in the soil, nodules are not evenly distributed on the roots, but appear on the parts of the roots nearest to the surface of the ground, and decreasing in size and number as they go down. This freedom from infection on the lower roots is due, Hiltner claims, to the immunity brought about by an early infection of the upper ones. When for any reason, nodules do not appear on the upper roots, they form later on the lower ones. A general distribution of the nodules results from inoculation after the growth of the plant is well advanced.

The location of nodules is independent of their oxygen requirements. The formation of nodules ceases only because a larger number would disturb the balance between bacteria and plant. It is not difficult to induce them to form on the deeper roots if those only are inoculated.

It is useless to inoculate open fields with nodule bacteria, unless the soil is free from them or contains only forms of lesser virulence than the ones used for inoculation. It has been repeatedly demonstrated that soils free from nodule bacteria do exist. Therefore Mazé's claim that bacteria are present but lack the necessary developmental conditions seems unjustified. Equally unjustified are the opinions of those who leave out of consideration the question of virulence.



It does not seem impossible to Hiltner, that the virulence may be increased beyond that naturally reached, by cultivating the bacteria for a long time on root extracts of increasing concentration, since it has been already demonstrated by Nobbe and Hiltner that repeated culture on gelatin containing extracts of the green parts of the plant increases the virulence of the bacteria materially.

The nodule bacteria are probably drawn to the root-hairs by chemically attractive excretions whose nature is not yet determined. They appear to be specific for each species of legume. In general they seem to be organic acids and acid phosphates. These are the substances which according to the convincing experiments of Stutzer cause the transformation of bacteria into bacteroids. This transformation takes place within the nodules as soon as the enveloping slime is dissolved by these acids, leaving the bacteria unable to penetrate further. Only when pea plants grown in a nitrogen-free solution become weakened, and secrete less protective substances, bacteria of poor virulence can penetrate the root-hairs, but these produce inactive nodules.

Nodule bacteria which have penetrated into the roots may be absorbed by the plant. Plants inoculated with lupin bacteria obtained from Höchst failed to produce nodules, although sections of the roots a few days after inoculation contained groups of bacteria. Further investigation showed that these had completely broken down into tiny microsome-like bodies, when they could be demonstrated at all. Although obtained from lupin nodules these were probably not genuine lupin-bacteria. Under similar conditions Hiltner's own isolations readily induced nodules and behaved differently on gelatin, *i. e.*, grew less vigorously.

Conditions of nutrition affect nodule formation. Solutions or soils poor or lacking in nitrogen are more favorable than those containing this element. Yet it only needs bacteria of a higher degree of virulence, adapted to the species, to infect even the best nourished plants.

Salt-peter prevents the formation of nodules. That this is not due to the abundance of nitrogen supplied as was formerly supposed, but to a directly injurious effect on the bacteria, is shown by the following experiments:

Inoculated pea plants remained free from nodules when grown in a liter of nutrient solution containing 5 mg. nitrogen in the form of salt-peter, while in a similar solution free from nitrogen abundant nodules were formed. The same results were obtained with *Robinia* and *Alnus*. It seems impossible that such a small amount of nitrogen should completely repress nodule formation by stimulating growth of the plant. The salt-peter must act, therefore, not by helping the plant, but by injuring the bacteria. Small doses of salt-peter are soon absorbed by the plant and then nodule formation sets in. Only when plants are transferred every 2 or 3 days into fresh solutions containing salt-peter do they remain permanently immune.

In quartz-sand cultures the effect of salt-peter was more marked than in mixtures of sand and soil though not nearly so great as in solutions, but 50 mgs. of nitrogen in the form of  $\text{KNO}_3$  to 1 liter of sand did not completely suppress nodule formation. The nodules remained small but bacteroid formation proceeded very rapidly. Salt-peter increases bacteroid formation extraordinarily, not only in the nodules but also in fluid cultures of the bacteria.

Within certain bounds, the more sandy the soil the greater is the injury caused by fertilization with salt-peter. As the most vigorous plants were found in rich humus soils where nodule formation was little suppressed by the salt-peter, it is evident that the suppression of root-nodules is not due simply to the invigoration of the plants by salt-peter.

Other nitrogenous compounds, such as ammonium sulphate had much less effect on nodule formation and extract of horse manure was entirely harmless.

The activity of the nodules begins to be visible in the parts above ground only when the available nitrogen in the soil begins to be exhausted. In nitrogen-free soil, seedlings must draw their supply from the seed. As this supply is exhausted before the activity of the nodules begins, a period of hunger intervenes, during which the leaves become strikingly yellow, and the growth of the plant almost ceases. Hellriegel and Wilfarth observed this and Hiltner has repeatedly confirmed it. A small amount of nitrogen placed in the soil, especially stable manure, will tide the plants over this stage, but a dose larger than that actually required for the development of the nodules hinders nitrogen assimilation. For the same reason on soils containing nitrogen a mixed growth of leguminous and non-leguminous plants is believed to be better than unmixed growths since the non-legumes will quickly use up the nitrogen, and the legumes under such conditions will come into activity sooner.

Hiltner thinks from the foregoing that the Leguminosae strongly influence the process of nitrification in the soil.

In salt-peter solutions, the root-hairs do not curl, showing that the bacteria are not able to penetrate. Yet the bacteria are not killed, for they succeed in penetrating after the salt-peter has been absorbed by the plant.

The immunity of the plant afforded by already active nodules appears to be due to an excretion from the bacteroids.

The view that the nitrogen-fixing activity of the nodules is due to an enzym excreted by the bacteroids was first advanced by Stoklasa, who said that lupin plants from which nodules had been carefully removed, continued to fix nitrogen. His results were not confirmed by Hiltner who doubted whether the roots remained free from nodules. In Hiltner's own water-culture experiments, *Robinia* plants, when freed from numerous active nodules, lost their power to assimilate free nitrogen shortly afterwards, but soon formed new nodules where the others had been. The same results were obtained with *Alnus* several years running. Uninoculated plants in nitrogen-free solutions plainly suffered from hunger. A sudden greening of the younger leaves followed the formation of a few nodules from spontaneous infection. When these were removed, starvation again set in until other nodules appeared. Stoklasa's conclusion must, therefore, be regarded as wholly erroneous.

Nevertheless Hiltner agrees with Stoklasa that the bacteroids do secrete an enzym-like substance which is absorbed by the plant and used as food. This substance may be seen, especially in glycerin mounts, in the form of greenish, spherical bodies of varying size, behaving like soluble albumen. He thinks that it is this substance which affords immunity by penetrating to all parts of the roots. It has not yet been determined whether it is identical with that contained in the slime of the bacteria, which exerts such a peculiar influence on the membrane of the root-hairs.

Root-nodules are active only under favorable conditions of temperature and moisture—conditions above those required for the plant. Hiltner found that beans planted in a mixture of sand and earth and inoculated in May, showed results by the end of the thirty-third day and a very strong growth by the end of September. The same experiment started the last of August produced plants that in 25 days were plainly suffering from nitrogen-hunger, which they did not overcome in spite of the presence of numerous large nodules. These nodules, therefore, remained wholly inactive during the unfavorable weather of September and October. On the other hand check plants in nitrogenous soil continued moderate growth to the end.

Different varieties of nodule bacteria may produce nodules varying in size and form on the same species of Leguminosae. Nobbe produced on *Robinia*, nodules like those of the pea by inoculating with *Pisum* bacteria. Hiltner states that he has obtained irregular forms of nodules on *Acacia lophanta* by inoculating with bacteria from pea or locust.

By altering his nutrient media, Hiltner states, he has obtained a better growth of bacteria than formerly. Cultures of lupin-bacteria obtained from the Farberwerken at Höchst formed luxuriant colonies but were not virulent. This was not due to loss of virulence from cultivation on artificial media. The bacteria were probably taken not from active nodules on the tap-root but from nodules on side roots caused by unadapted forms, and hence weak in virulence from the start. On this subject Hiltner says:

"In the future, therefore, when we wish to obtain really active, virulent, inoculating material for the lupin, we must take the cultures from nodules which appeared as early as possible, and give evidence of this by the fact that they are situated on the main root. Also, with all the other Leguminosae, it would be a great mistake to secure pure cultures from unselected nodules, or even from such nodules as occur deep down and at the ends of lateral roots."

The studies made on the nodules of leguminous plants of many species kept in botanical gardens do not hold good in all situations, where plants are subjected to varying conditions of soil, fertilization, virulence of bacteria, and climate. Thus the soy-bean which produced no nodules in Germany unless inoculated with soil from Japan, in France regularly produced nodules. On the other hand, *Phaseolus* which in Germany produces such large numerous nodules and seems to be most subject to infection with non-active bacteria, forms only small ones in France.

Hiltner is inclined to believe that the size of nodules is influenced by the rapidity with which the plant changes the bacteria into bacteroids. When this process is rapid, the nodules remain small but active. When it is slow they grow large and inactive.

Many have thought that the benefit to the plant was derived by the absorption of the bacteroids, since at the time when fruit was ripening the nodules were emptied. Nobbe and Hiltner claim, however, that this is not true. They found the greatest activity at a time when no sign of this emptying had appeared. Besides this, the amount of nitrogen assimilated by the nodules of a plant during a season exceeds a hundred fold that contained in the nodules. Hiltner thinks that the bacteroids are not absorbed, but simply regain their original form when the sap gradually loses the properties which transformed them into bacteroids in the first place, and that only such bacteroids are dissolved as had been so thoroughly changed as to lose the power of retransformation.

In 1902 Peirce in California published a monograph on the root-nodules of Bur clover. He stained by means of Flemming's stain, followed by Ehrlich's method of staining cover-glass preparations of bacteria. The sections, which must be left in the gentian violet for a minute or two only, were then placed a half hour or longer in Gram's iodine solution to differentiate the bacilli and the infection threads from the cytoplasm. He states that one minute is usually long enough in the gentian violet.

They are then rinsed in water and placed in the iodine solution. After washing this off they were allowed to remain one or two minutes in the orange G. They were then washed in absolute alcohol as long as gentian violet came off abundantly or needed to be removed. They were cleared in clove oil, this being preferred to xylol. He states that the bacteria were distinctly differentially stained in the tissues.

In the Bur clover the proportion of root-hairs attacked naturally by the nodule organism was estimated to be about one in a thousand. He, however, was able to confirm Miss Dawson's statement respecting the abundance of infected root-hairs when the roots of legumes were subjected to special methods of infection, *e. g.*, placed on damp filter paper under a tumbler, and watered with water containing the bacteria in suspension, nearly every hair on a root in some fields of the microscope was found to be enlarged and twisted at the end, and showed the beginning of an infection thread. A striking characteristic of the infection of the root-hairs is the curvature of the hair about the infected portion (fig. 21). It is believed that the organism dissolves or softens the walls of the root-hair and thus enters. A wound is not necessary. In fact, wounded root-hairs lose the power of curvature displayed so strikingly in the ones showing the infections. The curvature is unquestionably due to the presence of the bacteria.

"The bending is the evident response to irritation." \* \* \* "Since the majority of infected root-hairs show the bending at or near the tip, \* \* \* we may infer that the bacteria enter uninjured hairs which are able by growth-curvatures to respond to mechanical or chemical stimuli." \* \* \* "Having entered the root-hair by softening or dissolving a small portion of the cell-wall, and moving or growing through this, the tubercle bacteria multiply rapidly, forming a thread-like zoogloae from the infection spot along the hair into the epidermal cell of which the hair is a branch. From the epidermis the infecting zoogloae grows fairly straight into the underlying cortical parenchyma." \* \* \* "The direction of the infection thread—which is solid, and is incorrectly termed infection 'tube'—is too regular not to encourage one to suppose that the course of the growing strand of bacteria is determined by attraction exerted by the host-cells upon the bacteria. This then is chemotropic growth of the strand or, if the bacteria are motile in the cells, chemotactic movement of the bacteria. The course of the thread is toward the conducting tissues of the host." \* \* \* "The growth does not extend into the central cylinder and the conducting tissues, so far as I have seen. Instead, in the layer of cells just outside the endodermis of the root, division takes place in the cell into which the infection thread has penetrated and in the cells adjacent to it. The daughter-cells grow, repeated divisions and growth follow, and there arises a conical mass of cells which are somewhat larger, and which contain more protoplasm than the adjacent cortical parenchyma cells. This conical mass is the young tubercle. At first all of its cells are meristematic, but later the divisions become more and more limited to the cells near the rounded apex of the blunt cone. Thus a regular cambium is differentiated in the tubercle. This cambium \* \* \* lies near the tip of the tubercle, and forms a bowl-shaped or shallow thimble-shaped layer."

"The growing tubercle pushes out the overlying cortical parenchyma and epidermis, forming an increasing swelling on the side of the root. Cortical parenchyma and epidermis, at least for a time, nearly keep pace with the growth of the tubercle. Thus, although the cortical cells are compressed somewhat, the epidermis is not ruptured, and the tubercle does not burst out of the side of the root as a lateral root does."

Morphologically, in origin the nodules are indistinguishable from the nascent lateral roots. In subsequent growth they are more and more dissimilar.

"Morphologically, then, the root-tubercles are lateral roots." \* \* \* "The bacteria in the infection thread, which grows through the root-hair and the cortical parenchyma cells of the root to the pericambium layer, multiply, but they multiply most rapidly in the infected cells farthest from the surface of the root. New threads form, which grow out into and infect the cells of the mass of new cells composing the embryo-tubercle. Thus a majority of the cells in the young tubercle contain bacteria."

"Though infected cells do divide, they probably divide less often than the uninfected cells." \* \* \* "The infection of the daughter-cells composing the embryo-tubercle is accomplished by branching infection threads growing in fairly straight lines radiating from the base of the tubercle. In this way the cells near the base of the growing tubercle are most infected, those near the tip least."

The meristem continues to form new cells between itself and the cells containing bacteria and infection threads. When, however, he imprisoned the nodules in plaster of Paris, the meristematic cells also were infected.

The infected cells became enlarged and in their definitive condition are said to be from half as large again to twice as large as the uninfected cells. There was less difference in size of normal and infected cells in nodules imprisoned in plaster casts.

The infected cells are thin-walled and contain only one large vacuole. This is not traversed by

cytoplasmic strands. The quantity of the bacteria may vary in infected cells, and there is a corresponding variation in the appearance of these cells. Normal cells contain starch-grains. Cells with no bacteria contain many starch-grains. Those containing a small number of bacteria also contain some starch-grains, the number being in inverse proportion to the number of bacteria.

Peirce's study of the appearance of the tissue indicates beyond doubt that the nucleus and cytoplasm are seriously injured by the presence of the bacteria and finally destroyed (fig. 31). He looks upon the organism as beyond question a parasite. He says that the direction of the growth of the infection threads can not be determined by the oxygen or the nitrogen of the air, for if this were the case, the strands of bacteria would be found extending toward the periphery of the tubercle in all directions, which is not the case. "Not only do the infection threads run definitely toward the growing-point of the tubercle; they also grow toward the nucleus of each cell (fig. 22) which they enter." Even where at first they seemed not to grow toward the nucleus a study of serial sections showed that such was the case, branches in sections above or below being given off toward the nucleus.

"Microtome sections, differentially stained, as before described, of carefully fixed growing tubercles of the species of leguminous plants which I have especially studied, show that in most cases the infection threads run definitely toward the nuclei of the tubercle cells." \* \* \* "When infected cells contain any considerable number of bacteria, they cease to be able to divide." \* \* \* "The presence of the tubercle bacteria is not beneficial to the cells which contain the bacteria." \* \* \* "One point more needs to be made clear. Miss Dawson says that it is difficult to conceive how such strictly aerobic bacteria as these can flourish in the cells of such compact tissue as composes the tubercle. This difficulty is of her own conceiving, for do not the cells of the tubercles respire and are they not necessarily supplied with oxygen for respiration?" \* \* \* "Unless we are to imagine anaerobic respiration for these cells, it is unnecessary to assume it for the bacteria which infest them."

The following is a synopsis of the long paper by Hiltner and Störmer, published in 1903:

The "nitragin" inoculations having discouraged growers because very often the results were not what had been anticipated, additional seed and soil inoculations were undertaken and good results were often obtained, care being taken to use bacteria which were more active than those already in the soil. Experiments since 1900 have shown, however, that failure may be due not only to poor virulence but also to conditions which prevent the penetration of the bacteria. In inoculations of pure cultures mixed with soil, success depends on chemical and physical soil conditions favorable to the development of the bacteria. In inoculations by moistening the seed, failure is probably often due to the fact, discovered by the authors, that a substance dissolved out of the seed-coat during germination exerts an injurious influence on the bacteria.

In spite of many failures, and in spite of the widespread opinion, expressed by Gerlach, that pure culture inoculations are of no value since sufficient bacteria already exist in the soil, the authors are convinced that soil inoculation has a future, since nitragin has given results safely beyond the limit of error in a sufficient number of instances to refute the opposing theory. One of these favorable experiments was that of Loges who obtained an increase in crop of 124 per cent with field beans, 46.7 per cent with peas, and 400 per cent with vetches. In this case the sandy field used had not borne any legumes, except lupins, for a number of years. Seed inoculation was used. The favorable result here was probably due to the fact that the seeds were soaked 24 hours before they were inoculated, and hence the bacteria did not have to encounter the injurious substance in the seed-coat. The inoculated seeds germinated more quickly than the uninoculated ones, which had also been soaked. Although this was undoubtedly independent of inoculation it gave opportunity for early and successful penetration of the bacteria into the roots.

When rainy weather follows seed-inoculation, the bacteria may be washed from the seeds to places where they will not be affected by the injurious substance in the seed-coat and yet where they can reach the roots. When sufficient moisture is present, similarly, favorable results may be obtained by the otherwise uncertain method of direct inoculation of the soil.

Dietrich obtained good results with blue lupin by inoculating the soil 12 days after sowing, when the plants were well started. Rainy weather favored the spread of the nitragin through the soil. His crop from inoculated soil was 65 per cent greater in green substance and 92 per cent richer in nitrogen than that from uninoculated soil.

The inoculation of the field by strewing inoculated soil can not be recommended universally because results are too dependent upon the weather. If continued dry weather follows the sowing, the probability that the bacteria will reach the roots diminishes daily.

In some cases where the inoculated fields showed an increase in crop of from 10 to 24 per cent over uninoculated ones, the difference in stand was scarcely noticeable. This fact may explain many seeming failures where the results have been judged only by appearances.

Successful results with serradella were obtained by Luberg on dry sandy land from seed-inoculation which was followed by unfavorable weather. This was probably due to the fact that serradella seeds are protected by a very heavy seed-coat and hence do not need the protective substance fatal to bacteria.

Inoculation by strewing infected soil seems relatively a better method than seed inoculation, yet it needs improvement. Only when the soil offers to the bacteria favorable conditions for growth, or when its bacterial content does not act unfavorably on them, as for example on moors and marshes, can inoculation with soil either before or after sowing be used with any certainty of success.

Tacke obtained remarkable results with peas on newly cultivated moor-land. The crops resulting from seed-inoculation were 282 per cent greater, from soil-inoculation 384 per cent larger than crops from uninoculated land or seeds. Von Feilitzen obtained like results in Sweden on similar new land. His seed-inoculations gave an increase of 55 per cent straw and 116 per cent seed. This author also found an advantage in the use of nitragin over that of natural earth inoculation, as the latter caused a heavy growth of weeds fatal to the crop.

Wollney concludes from his experiments with peas, field beans, white lupins, scarlet clover, and serradella, that nitragin can be successfully used only on newly cultivated soils or on soils which have borne no legumes and are sandy without humus. On soils containing humus, virulent root-nodule bacteria are already present and inoculations are useless. He bases this opinion on the fact that nodules occurred on the roots of all the plants sowed, yet only the peas showed increased growth. In all these cases, according to Hiltner, results would probably have been favorable if inoculations had been made with organisms more virulent than those present in the soil.

Schulze on the other hand, like Kühn, expresses a belief in the future of nitragin. He claims that the few cases which have been successful prove that nitragin can work, and that what is now needed is elimination of unfavorable factors and improvement in the methods of application.

The excessive claims made for the earlier nitragin against which, however, Nobbe and Hiltner protested repeatedly but in vain, have undoubtedly had much to do with bringing inoculations into disrepute. Hiltner thinks, however, that pure culture inoculations will in the future, in spite of all obstacles, win a place in practical agriculture, and he has desired especially because of his connection with the old nitragin to help on in all possible ways this view which he has never ceased to maintain.

From 1900 on, only pure cultures were used by Hiltner.

Although a former experiment by Loges gives favorable results from soaking the seeds before inoculation, numerous experiments by Hiltner have shown that this method is not to be generally recommended, since seeds so treated, although germinating readily, are especially liable to rot in the soil.\* Thiele failed to get a stand with either peas or beans which had been soaked 24 hours before sowing since all the beans and most of the peas rotted in the ground. He attributed this to dry weather prevailing before and after sowing. Hiltner, however, thinks the failure due to the destructive action of soil organisms.

Dr. Böhme, on the other hand, obtained strikingly favorable results with yellow clover by soaking the seeds in a quantity of water containing the bacteria into which was sifted a little fine earth, and sowing after two days when they had absorbed all the water and were dried out. The inoculated plants attained a height of 160 mm. with deep green leaves 15 mm. broad, while the uninoculated plants were only a few centimeters high with pale green leaves scarcely 4 mm. broad.

In 1901, 59 experiments were carried on for Hiltner by 31 different men according to the following directions: Soaking seed previous to sowing must be avoided; the seeds should be thoroughly wetted with the inoculating fluid, the excess of moisture removed by a sprinkling of dry sand, after which the seed drill can be used for sowing. As at this time the injurious action of the substance contained in the seed-coat had not been discovered, this factor could not be taken into account. Hence in none of the experiments of 1901 was the method used which is now considered necessary. Yet the collective results of the year's work justified great hopes for the future.

One group of 13 experiments was eliminated by the fact that dry weather prevented germination or destroyed the young plants. In another group of 21 experiments the effects of inoculation were either imperceptible or doubtful. For example, in one experiment with beans, Braun states that 6 weeks of drought caused the plants to wither before they had bloomed. At the beginning of August, after a good rain, growth again set in and the crop reached maturity, but no effect of the nitragin was observable either in foliage or fruit. In another case while inoculated serradella gave 13 per cent increase in dry substance, inoculated red clover showed an equivalent loss. Concerning these results, Hiltner says:

"We also are of the opinion that results can not be obtained under all circumstances by inoculation; but for the present we cling fast to the hope that in the future, after further improvement of

\*Consult Hiltner's paper. Arb. a. d. Bio. Abt. f. Land. u. Forst. a. k. Ges., III Bd., Heft. 1, Berlin, 1902.

the inoculating materials and the methods of inoculation, the cases where results of inoculation are completely lacking will be exceptions."

The third group of 25 experiments all showed positive results from the inoculation. One of these reported by Gerlach was peculiar in that while the green substance showed an increase in weight, the weight of the dry substance fell below that of the uninoculated plants. These plants were subject to a long drought during the middle period of their growth. Hiltner observed the same phenomenon with soy-beans which ripened late, though under favorable weather conditions the growth of the soy-bean is increased by the presence of nodules.

In one set of experiments it was noticeable that the small, seeded species gave good results from seed inoculation while with large seeded species such as peas and lupins, the results were either doubtful or negative. This was probably due in the case of the large seeds to the inhibiting action of excretions from the seed-coat.

Another grower obtained a gain of 50 per cent in straw and seed with soy-bean. Hiltner who saw and examined the mature crop early in October, states that the roots of the uninoculated plants were free from nodules, while nodules were formed on about 50 per cent of the plants in the inoculated plots.

In another case where poor soil was used the inoculated vetches were backward compared to the uninoculated; later, however, they outstripped them with a more luxuriant green and a denser mass but the weeds also seemed to grow better there.

Director H. Rose obtained striking results with serradella and yellow lupin. The seeds were carefully inoculated and sowed in good weather. The field used was a new, light sandy, rather moist heather land, which had borne previously only rye, fertilized with lime-kainit-phosphate and Chile saltpeter.

For this experiment 500 kg. of Thomas-meal and 600 kg. of kainit per hektar was applied in the spring. The seeds were sown on May 11. The weather was very dry, only two rains occurring during the whole summer. Growth which began slowly in the serradella took on new life late in June in the inoculated plots, so that early in July while the plants of the uninoculated plots were barely 10 cm. high, unbranched, yellow, and sickly with absolutely no nodules, those in the inoculated plots were 20 to 30 cm. high, branched so as to form a close mat and with roots full of thick, watery nodules. The results with lupin were similar but less marked. The harvest of serradella (*Ornithopus sativa*) is given below:

Plot	Green weight.	Seed.
I. Uninoculated, per hektar....	5,300 kg.	410 kg.
III. Uninoculated, per hektar....	6,800 kg.	470 kg.
II. Inoculated, per hektar.....	12,250 kg.	680 kg.
IV. Inoculated, per hektar.....	11,750 kg.	615 kg.

Hiltner also saw the crops of Tacke, Director of the Moor Station at Bremen, just before they were harvested, and states that of the uninoculated plants, most were suffering hunger. There were yellow lupins, blue lupins, scarlet clover, and serradella. A few plants showed by their growth the presence of nodules, but others (yellow lupin and scarlet clover) possessed large nodules which were completely inactive. On yellow lupin, seed-inoculations gave no improvement over the check but inoculation with infected soil gave a luxuriant crop, 80 cm. high, all plants having nodules which were arranged in rows mostly on the lateral roots.

Blue lupin gave similar results. About 30 per cent of the uninoculated plants formed on the deeper roots a few nodules which exerted little influence on the growth. Seed-inoculation gave 35 per cent of nodule-bearing plants which, however, did not show any benefit from the nodules, which were on the lateral roots and hence due to spontaneous infection. Inoculation with infected soil produced luxuriant growth, all the plants bearing numerous nodules. Inoculation with natural earth gave equally good results.

Uninoculated crimson clover was extremely scanty and sickly in growth in spite of nodules on the roots of all the plants. Here seed-inoculation gave the best results, with numerous nodules on all parts of the roots.

Uninoculated serradella was also sickly. Seeds which were soaked before sowing gave by far the best results.

These experiments brought out the fact that in all cases uninoculated plots, or plots inoculated with pure cultures, were almost entirely free from weeds, while those on which natural soil inoculations were made were overgrown with weeds, especially wild spurry which is hard to eliminate. This observation has been repeatedly made by Tacke.

The following table gives the harvested crops in kilograms per plot, omitting fractions less than 0.5 and adding those more than 0.5:

	Uninoculated.	Seed inoculated.	Soil inoculated.	Soaked seeds.	Natural soil inoculation.
Yellow lupin .....	112	89	385	213	133
Blue lupin .....	40	37	150	93	162
Crimson clover .....	0	244	112	14	17
Serradella .....	41	54	41	160	42

In summing up the 46 experiments of this year (1901) at Bremen it may be said that 54 per cent were favorable, some extraordinarily so, in spite of the many unfavorable conditions. This result certainly refutes the claim that inoculations in the open give recognizable results only in quite isolated cases. It is also plain that results are the more certain the less often the land used has borne the species of legume in question. The method of inoculation is of the utmost importance and must be selected with reference to the soil and the species of legume.

In the experiments at Dahlem, *i. e.*, under Hiltner's direct supervision, *Soja hispida* was used exclusively, since it is not there subject to spontaneous infection, but forms, when inoculated, large easily counted nodules in which, at a temperature suited to the Soja, active nitrogen assimilation takes place. In 1901 experiments were made to determine how long nodule bacteria remain active in the soil. Plots were used which had borne soy-beans the previous year, some uninoculated, others inoculated at that time.

Both yellow and brown seeded varieties formed an average of 75 nodules on all plants grown in previously inoculated soil. Out of 356 plants examined none had less than 50 nodules. From this it is evident that the nodule bacteria which had wintered in the soil must have retained a high degree of virulence. Hence, when nodule bacteria prove virulent one year, they are also virulent for the same crop the next year, and even increasingly effective. This conclusion agrees with the results of growers who have found that one inoculation suffices for a series of years where the same crop is cultivated, and that further inoculation in such cases is useless.

On soil which had been inoculated with Soja earth and cultivated in oats in 1900, the Soja-beans of 1901 were sparingly infected. These nodules were due, however, to an infection from neighboring plots rather than to bacteria which had persisted in the soil, as was shown by comparison of the two varieties in the four oat plots of 1900. The bacteria, therefore, can live from one year to the next in the Dahlemsoil only when the legume to which they belong is at hand so that nodules are formed. They are able to draw nourishment from the decaying roots, and, when these are gone, to hold their own with the other soil organisms for a limited time only.

Another experiment in 1901 was made with soy-beans on land that had in 1900 borne various crops. In the uninoculated plots the average number of nodules varied from 0 to 1.2. Most of the plants were free. On the contrary in the plots where *Phaseolus* had grown in 1900, 41 per cent of the plants bore nodules. The results when inoculation took place 6 weeks before sowing were very moderate (3 to 14 nodules), and no difference could be observed between the plots which in 1900 had borne legumes and those which had not. A 30 times larger amount of inoculating material caused a larger number of nodules, but even then the number did not equal those formed when inoculation took place at the time of sowing. What caused this failure, when 6 weeks elapsed between the inoculation of the soil and the sowing of the seed, is not certain. At any rate it was not due to lack of moisture, as moisture conditions were excellent.

Experiments with soy-bean to determine the best method of inoculation again showed the action of an injurious substance in the seed-coat. Inoculation made by strewing Dahlem sand which had been moistened with a pure culture produced plants which were absolutely nodule free. The most favorable results were obtained by germinating the seed and then inoculating just before sowing. These facts indicate that seeds which have been soaked exert no longer an injurious influence: This decreases as the germination progresses. The inoculation with pure cultures in all the experiments showed itself equal to natural earth inoculations and in most cases was superior to the latter.

The method of inoculation by strewing earth previously infected with pure cultures proved beneficial only on cultivated moor soil, or in moist weather, or with seeds which germinate very rapidly.

The method by seed-inoculation may entirely miscarry owing to destruction of the bacteria by poisonous substances extruded from the seed-coat during germination. This danger is much greater with large seeds, such as lupins, peas, and soy-beans, than with small seeds, but may occur with the latter, *e. g.*, clover seed, if the soil is very dry and germination slow.

The surest method of seed-inoculation is first to swell the seeds and then inoculate them. The soaking of the seeds must, however, never take place under water but in moist sand.

The name bacteroid, it is said, originated with Brunchorst (1885) who implied thereby that these peculiar bodies while they resemble bacteria in size, shape, and staining properties have really nothing to do with bacteria. Beyerinck, by cultivating them on artificial media, showed their true bacterial nature. Prazmowski was the first to produce nodules by inoculation with pure cultures (1890).

Frank then abandoned his earlier views and described a micrococcus which he had isolated from the nodules and which he named *Rhizobium leguminosarum*. This he claimed differed in form and behavior on gelatin from Beyerinck's organism, and was the true cause of the nodule formation. He claimed that these bacteria were taken up by the bacteroids which he considered albuminous bodies, and released only when the albumen was absorbed by the plant. He termed the mixture of bacteria and plasma Mykoplasma. Afterwards, Frank admitted that the bacteroids developed from the nodule bacteria but claimed that they were only involution forms serving as storehouses for the albumen which the plant absorbed. This view has prevailed until recently. Hiltner maintains that this is a false idea. They are not involution forms. Frank does not explain whence bacteroids obtain their albumen when plants are grown in nitrogen-free sand. Further, according to Hiltner, the increased growth of the plant takes place before any absorption of bacteroids occurs and the amount of nitrogen fixed by the plant during a season may exceed by 100 times that contained in the nodules.

Hartleb, it is said, succeeded in producing bacteroids in artificial media, and attributed the alteration to the action of phosphoric acid. Hiltner does not agree with this latter view though he states that there is no doubt that bacteroids may be produced by artificial means, as he himself obtained them with root-extracts from legumes.

Hiltner has repeatedly opposed the idea that the bacteroids are involution forms, and has expressed the opinion that they are to be considered as sporangia. Hartleb's conclusions agree with this (1900), and a similar idea is expressed by Winkler who termed the bacteroids, bacterioplasts.

Hiltner undertook to determine the nature of the injurious action exerted by the substance contained in the seed-coat of legumes. To this end nodule bacteria were cultivated in water in which seeds had been soaked and to which various nutrient materials had been added.

The clear yellowish liquid obtained by soaking peas for 24 hours was filtered, and clouded slightly when an equal volume of alcohol was added, giving a slight precipitate. Tests for tannin gave negative results. Corrosive sublimate caused a flocculent precipitate in a 50 per cent alcoholic solution. A characteristic precipitate formed when to the solution were added three parts of alcohol and a concentrated platinum chloride solution, or, first, platinum chloride and then the alcohol. This precipitate was also obtained in the solution after it had been heated for 20 minutes at 120°. A second watery extract from the already soaked peas, which was also somewhat yellowish did not give this reaction.

Cultures were then made by putting a loop from a 3 to 4 weeks old gelatin culture into sterile water and transferring 0.5 cc. of this to 10 cc. of concentrated watery extract from lupin seeds and also into a threefold dilution of the same. These bacteria were taken from nodules on *Vicia villosa*. A test after 24 hours with carbol-fuchsin showed that bacteroid formation had begun in greater amount in the dilution than in the concentrated solution. After 2 days in both solutions a rapid growth of exclusively rod-shaped bacteroids began. Check cultures in tap water contained practically only normal bacteria. In 13 days the concentrated solutions were very heavily clouded and had formed a slimy precipitate. In the dilutions there was a somewhat weaker growth. In both there were only bacteroids. These were large with numerous vacuoles.

Bacteria cultivated from pea nodules and locust nodules gave a similar growth in 2 per cent solution, but the bacteroid-like bodies which varied greatly in form and in vacuole formation and thus suggested involution forms, often seemed to break up into fine granules. No multiplication was observable. This modification is traceable to the excretion from the seed-coat. These he regards as true involution forms.

In concentrated solutions, however, and in dilutions to which bouillon was added, true bacteroid formation occurred (with pea bacteria), the individuals were all alike, and there was no suggestion of a pathological origin.

Water in which very hard-shelled locust seeds had been soaked did not cause bacteroid formation with locust bacteria as had been expected. The water from soja-bean seeds caused a greater formation of bacteroids from soja-bean bacteria than did the water from peas upon pea bacteria.

From his experiments Hiltner concludes that several substances are present in the extract from the seed-coats: a pectin-like substance to which the formation of bacteroids is due, and which, instead of injuring the nodule bacteria, aids their growth; a further albuminous substance which is likewise favorable to the bacteria; third, an injurious substance excreted much sooner than the others in the form of a potassium salt which may be precipitated by platinum chloride and which with other



unknown substances together cause plasmolysis and death of the bacteria when they reach a certain concentration and this prior to the extrusion of the pectin-like substance.

The most important difference between the ordinary nodule bacteria and the bacteroids is not in form, since there are many unbranched bacteroids, but in their plasma contents which is vacuolate and otherwise unlike that of the ordinary rods. The real difference, therefore, is in the processes through which the plasma passes. This phenomenon was observed in solutions containing 1 per cent grape-sugar. The bacteria from soy-beans gave striking results. Almost without exception the slender, feebly staining bacteroids showed in one week lateral, generally spherical but sometimes broad-based, projections consisting of strongly staining plasma. Occasionally these projections occurred at both ends. The projection or outgrowth was strongly differentiated from the rod by tincture of iodine: The rod stained bright yellow, the projecting plasma red brown. In living uncolored preparations these projections were highly refractive bodies. They were not destroyed by dilute potash solution or dilute sulphuric acid, nor influenced in their ability to stain with carbol fuchsin. Staining alive in neutral red and eosin gave unsatisfactory results. With iodine potassium-iodide the projections stained mostly yellow while the rodlets remained unstained. To this projecting plasma Hiltner gives the name nuclear plasma in contrast to the part remaining within the rod which stains weakly with carbol fuchsin and which he calls cell-plasma or nutrient plasma. Similar observations were made on lupin bacteria.

After 2 weeks' growth the appearance of the soja bacteria was yet more striking. The two parts were still present and stained as before. From all appearances these bacteroids, except for a scarcely visible layer lining the walls, consisted of the same albuminous substances as is found collected in the vacuoles of the normal bacteroids.

After 49 days growth the nuclear plasma of the bacteroids from the bacteria of *Trifolium incarnatum* had broken up into normal bacteria. The same thing had occurred with those from *Trifolium hybridum* and *T. pratense*, and new bacteroid formation had begun. This process held true for all the bacteria of the *Vicia* group.

The bacteria of *Phaseolus vulgaris* showed, especially clearly after 3 weeks' growth, the breaking up of the much enlarged, strongly staining bodies into normal bacteria.

The following conclusions are drawn from these observations:

In 1 per cent grape-sugar solution bacteroid formation is rapid. They are distinguished from the normal rods by larger size and by a sharp differentiation in the plasma; one part, the germ-plasm or nuclear plasma, either pushing out as a spherical projection or gathering in definite spots within the bacteroid. Two groups are distinguishable, those which retain the form of rodlets when enlarging into bacteroids and on which the projections appear at the ends, and those which enlarge chiefly in breadth becoming spherical or pear shaped, and almost completely filled with the strong staining nuclear plasma: The rapidity of this process varies with the various nodule bacteria.

Cultures in solutions containing grape-sugar in concentrations of from 0.01 per cent to 5 per cent showed that, in general, growth is the stronger as the solution is weaker. The 2 per cent and 5 per cent solutions remained almost clear 4 days after inoculation. A concentration lying between 0.1 per cent and 1 per cent causes the most rapid bacteroid formation and plasma differentiation.

In salt-peter solutions the nodule bacteria from the pea made most vigorous growth in the stronger concentrations (1 and 2 per cent) but bacteroid formation was greater in the weakest solutions, *i. e.*, 0.1 per cent and 0.05 per cent. In these solutions after 3 days' growth a few isolated rods remained almost unaltered, while the bacteroids which were branched, and 10 times as long as broad, were collected in groups of a hundred or more. They stained uniformly, and while vacuoles were present they were not so highly differentiated as in the grape-sugar solutions. The soy-bean organism grew badly in salt-peter solutions and otherwise differently from the pea bacteria but with the formation of bacteroids. These were also obtained in asparagin water.

In 0.01 per cent to 5 per cent peptone solutions, the bacteria from soy-bean and *Vicia sativa* were totally unchanged after 4 weeks' growth, showing that this substance does not induce bacteroid formation. Growth occurred in the 5 per cent solution but was best in the 1 per cent solution. Soy-bean bacteria which grew poorly in salt-peter solutions did better than the vetch bacteria in peptone solutions.

In 1 per cent grape-sugar solutions which contained also 0.5 per cent to 2 per cent salt-peter the bacteroids of the pea showed a slight differentiation in their contents. Small, strongly staining granules appeared which at times seemed to lie on the exterior of the bacteroid. In general, also in solutions containing grape-sugar, the salt-peter caused very large, branching bacteroids with a net-like plasma. As the salt-peter in the weaker solutions was used up by the organism the effect of the grape-sugar became evident in the pushing out of the plasma masses which stained red brown with iodine and which were much larger than in pure grape-sugar solutions.

A solution containing 0.1 per cent asparagin showed astonishing results with the vetch bacteria,

*i. e.*, sproutings which stained strongly on nearly every rod and which developed sidewise often the whole length of the rod.

Asparagin in 1 per cent grape-sugar gave results similar to those of saltpeter and grape-sugar, that is the differentiation of nuclear plasma from cell-plasma took place only when the greater part of the asparagin had been used up by the bacteria.

From these results and similar ones obtained with peptone and grape-sugar solutions many of which are figured, Hiltner concludes that the differentiation of the plasma and the *Aussprossungen* connected therewith takes place only when the source of nitrogen is largely exhausted by the bacteria.\*

In his experiments with phosphoric acid, Hiltner failed to verify Hartleb's statement that only by the addition of alkaline salts of phosphoric acid is bacteroid formation induced in liquid media containing carbon and nitrogen. In Hiltner's carbon-free cultures the potassium phosphates were unable to bring about bacteroid formation. In cultures containing, in addition to the potassium phosphate, 0.1 per cent to 5 per cent grape-sugar, growth took place, but bacteroid formation was less marked than in the corresponding pure grape-sugar solutions. He concludes, therefore, that in such fluids the bacteroid formation is due exclusively to the presence of the carbon compounds and that the addition of phosphates, while it favors multiplication, retards bacteroid formation and the differentiation of the plasma. The rôle of the phosphate is solely that of nutrition. Saltpeter alone, of all the non-carbon substances tried, was able to cause bacteroid formation by itself.

The effect of ten other carbohydrates was tested in comparison with that of grape-sugar. One per cent sugar solutions were used (0.5 per cent with the pentoses) with and without 0.1 per cent asparagin. Laevulose was especially active in its influence on soy-bean bacteria. In 5 days the plasma had gathered into a spore-like body. Two weeks after the bacteroids were very large with outgrowths and a month later were immense bodies staining poorly with carbol fuchsin but well with tincture of iodine. All possessed very large, granular, honey-combed outgrowths which stained strongly.

Raffinose, cane-sugar, mannit, and galactose had the greatest effect on pea bacteria, while laevulose had here almost no effect.

*Robinia* bacteria grew well in cane-sugar and mannit, fairly well in laevulose and lactose but poorly in all the other solutions. Cane-sugar had the greatest effect and grape-sugar the least effect on bacteroid formation and plasma differentiation.

Tests with organic acids showed that these have a variable effect on bacteroid formations expressed by branching, plasma differentiation, and outgrowth. Hartleb's objections to Stutzer's statements are, therefore, untenable. Succinic acid was most active in its effect while citric acid was least so. The simultaneous addition of grape-sugar hastened the breaking up of the peculiar forms (outgrowths) developed from the bacteroids.

The most striking fact in bacteroid formation is the outpushing of the nuclear plasma, a process which occurs in the nodules as well as in artificial media. This process does not leave behind an empty membrane, but rather consists of a differentiation of the plasma into an out-pushing nuclear plasma and a nutrient plasma that remains behind. As shown by the power of a bacteroid to resume growth after this outgrowth has occurred, some of the nuclear plasma must remain within the rodlet, just as the outgrowth must contain some nutrient plasma.

The red-brown stain is not a reaction of the nuclear plasma as such, but rather of an unorganized substance arising from its activity. This may be easily separated from the plasma by different solutions and also may be worked over and used by it so that the red-brown color does not always appear when iodine is applied. For example, one finds very often in the same culture bacteroids with outgrowths which take this red-brown color along with those which stain pure yellow.

Prazmowski pointed out this plasma differentiation and considered the refractive red-brown substance as a peculiar form of albumen, formed under the influence of the plant. Frank's results also agree with these so far as the differentiation of the plasma is concerned. Frank further distinguished in the pea two sorts of nodules, one containing albumen, the other amyloextrin. Möller has shown, however, that this latter substance stains with iodine like glycogen and not like amyloextrin. Further evidence against the starchy nature of these granules lies in the fact that they do not swell in concentrated calcium nitrate and are not changed by boiling water. They are insoluble in cold dilute potash lye, cold or boiling concentrated ammonia, in hot ethyl alcohol and amyl alcohol, in ether, benzene or carbon bisulphide. This substance is not vaporized or otherwise changed by careful heating over the flame. It is easily dissolved by chloroform, acetone, glacial acetic acid, clove oil, and less easily by benzol. These reactions indicate that the ground substance is neither a carbohydrate nor albumen, but a fatty or waxy substance corresponding most nearly to cholesterolin, but not identical.

\*Similar extrusions from the cells have been observed by Kuntze and others in the lactic acid group of bacteria grown in whey, these bodies being Gram negative (see White and Avery, *Centralb. f. Bakt., II Abt.*, Bd. 25, 1909, p.165).

Frank in his first paper on the dimorphism of the pea nodules stated that the albuminous nodules contain 6.936 per cent nitrogen while those containing the "amyloextrin" only 4.828 per cent nitrogen in the dry substance.

Hiltner denies the existence of a dimorphism of pea nodules in the sense used by Frank, but says we might speak of a dimorphism of the bacteroids. He also takes issue with Möller that the end of the bacteroids in all nodules is fatty degeneration. Specimens examined by him showed plainly all degrees of transition between the two sorts of nodules described by Frank. The contents of normal pea nodules flow out readily when the nodule is cut, and are easily mixed with water. On the contrary the nodules in question which are distinguished outwardly by a somewhat darker color show in section a chalky consistency; even from cut cells the contents do not flow out into the water, but adhere so firmly together that it is difficult to separate the single elements from each other. Normal nodules, even when in decay, show no sign of this chalky consistency. The cells of the bacteroid tissue in these chalky nodules are filled with large starch-like grains which, however, stain red-brown with iodine instead of blue. An examination of these chalky nodules showed that almost without exception the septate mycelium of a fungus was present in the nodule or on that part of the root from which the nodule grew. The subsequent investigations at Dahlem verified the assumption that the aggregation of a fatty substance was to be referred to this fungus. Hiltner verified Frank's statement that these abnormal nodules are likely to occur on land where the pea has been cultivated several years. This may, he says, be due to early exposure of the roots to soil fungi, or to the lack of a physiologically important nutrient substance.

At Dahlem, in soil used for the first time for pea culture, often after 6 to 8 weeks' growth, in a portion of the plants, the upper leaves of pea vines formerly green and healthy took on a pale color and a cessation of growth occurred. In another such field sickly plants appeared among the healthy ones. Investigation showed that healthy plants possessed normal nodules while great numbers of abnormal nodules were found on the roots of the sickly plants, so that one could tell by the appearance of the plants before uprooting them, whether the roots bore normal or abnormal nodules. From these facts it is easy to explain why abnormal nodules form on the pea, for the pea is known to experience *Bodenmüdigkeit* very rapidly and even to fail on some soils in its first year of cultivation after passing successfully the earlier stages of growth. Hiltner considers this a valuable addition to our knowledge of the causes of soil sickness.

A close study of the waxy substances of bacteroids from artificial cultures, which substance is similar to that in the abnormal nodules, led Hiltner to agree with Möller in regard to its solubility but to disagree with Frank in that he found it unchanged by concentrated sulphuric acid. It can be shaken out of nodules or old cultures by means of chloroform and has rather the consistency of gutta-percha than of wax. The red-brown color caused by iodine is not a reaction of the waxy substance but of a soluble substance present with it. When sections of waxy nodules were left in water for 2 days the granules stained pure yellow instead of red-brown, without having otherwise changed in appearance. From his experiments Hiltner found no reason why the red-brown color should not be considered as a glycogen reaction.

A qualitative analysis of this waxy substance from abnormal nodules showed that it was absolutely nitrogen-free. This was at first a disappointing discovery since Hiltner had considered that the waxy substance, so constant in its appearance in artificial cultures and in abnormal nodules represented an accumulation of the products of nitrogen assimilation which the plants had lost the power to absorb. To test this point further, cultures of nodule bacteria from pea, soy-bean and *Robinia* were allowed to grow for 4 months in seven different nutrient solutions and then tested for increase in nitrogen. Though in suitable solutions the large outgrowths containing waxy inclusions had been formed, in no case was there a trace of nitrogen increase. These solutions contained 1 per cent grape-sugar, 0.02 per cent monopotassium phosphate and variable amounts of nitrogen in the form of peptone, asparagin and saltpetre—in all 50 experiments.

It is further interesting to note that while, ordinarily, nodule bacteria stain very little or not at all by Gram's method, the waxy outgrowths or germ-plasma of the bacteroids, arising in nutrient solutions containing carbon compounds, stain intensively by Gram soon after their differentiation. As this stage passes, the ability to retain the stain is gradually lost. The substance which holds this stain evidently passes largely into the bacterial slime for this stains readily while the almost colorless bacteroids show then only a few deeply colored granules which sometimes stick to their exterior.

The following observations were made on the soy-bean, grown in pots for several years.

In this plant the hunger stage lasts uncommonly long. The plants suffer from it even after numerous large nodules have formed on the roots. On plants showing nitrogen hunger for about 8 days, the bacteroids of these inactive nodules were small and only beginning to show plasma differentiation. In the nodules of other plants which the authors knew would in a few days show greening from the beginning of nitrogen assimilation, all the bacteroids possessed large, roundish

outgrowths like those which had been observed repeatedly in nutrient solutions. These outgrowths stained strongly with carbol fuchsin. However, when these plants actually became green there was no longer a trace of the outgrowths on the bacteroids. The outgrowths, therefore, formed when the plants began to hunger and disappeared as the nitrogen hunger stage passed away. From this it appears that nitrogen assimilation must stand in some sort of relation to the absorption of the outgrowths of the bacteroids which in active tubercles are continually formed but not easily demonstrated. Nitrogen assimilation is made possible not by the bacteroid formation per se, but by the process of plasma differentiation therein, in that from the nuclear plasma or through its action a nitrogenous substance is formed, the nitrogen for which is drawn from the atmosphere.

Bacteroid formation and nitrogen assimilation do not always go hand in hand. This showed plainly in the cultures of pea bacteria in saltpeter solutions previously referred to, where, though bacteroid formation took place, plasma outgrowths did not appear, no staining was obtained with Gram's stain, with iodine the color became yellow, not red-brown, and no nitrogen assimilation occurred. These circumstances explain the fact previously mentioned by Nobbe and Hiltner that nitrogen assimilation in Leguminosae begins only when the available nitrogen in the soil has been exhausted. This is true also in *Alnus*.

Hartleb's idea of the sporangial nature of the bacteroid is not new but originated with Brunchorst and Moeller and was confirmed by Hiltner prior to the appearance of Hartleb's paper. Later experiments by the authors have not altered this view. The bacteroids of the alder bacteria are even more strikingly sporangia-like. All observations point to the conclusion that in certain solutions and under certain circumstances the outgrowths from the bacteroids break up into pieces of varying size which directly or after further division may grow into bacteria or bacteroids. Some of Hiltner's figures suggest certain figures published by Gino de Rossi. The bacteroids within the nodules show less plainly their sporangial nature.

According to these observations the relation between host-plant and bacteroids is much more intimate than was previously supposed. In sharp contrast to the opinion that the nitrogen assimilation is concerned with an absorption of the bacteroids, Hiltner claims that his observations support the view that under normal circumstances this absorption does not take place, but rather that some substance produced by the nitrogen assimilation which goes on within the bacteroid is absorbed. This opinion is based on the fact that within the root-nodules the bacteria have a tendency to form sporangia to protect themselves against the influence of the host-plant, but that this does not succeed so long as the plant is active, because the indispensable building material obtained by nitrogen assimilation is constantly drawn away from them by the plant.

Beyerinck also has found that soil bacteria known as true nitrogen fixers (his *Granulobacter*, *Radiobacter*, *Aerobacter*) are able to use very little of the product of their assimilation, but that this is used chiefly by a species living with them (*Azotobacter chroococcum*), the nitrogen fixers (other than *Granulobacter*) being stimulated to action only by the symbiotic life.

The effectiveness of pure cultures of nodule bacteria depends on a number of factors, viz., genuineness, nutrition, virulence, and nitrogen-fixing power.

That true nodule bacteria should be used is self evident, yet that extreme care is required to obtain them is not fully realized. Nodules in which decay has begun may contain all manner of strange species and it is not out of the question that completely sound nodules may contain intruders. Beyerinck showed this to be the case. Though Hiltner did not find the same intruding species except in decaying nodules, he did find in 1902 another contaminating species in cultures from several distinct sources. This intruding species developed very slowly on gelatin compared with the true nodule bacteria, and differed from it quite materially in the appearance of its colonies. On the other hand it formed bacteroids and behaved very similarly to the nodule bacteria in grape-sugar solutions. This organism was not able to produce nodules and seemed able to penetrate the nodules only when the real nodule producer had prepared the way. An especially characteristic peculiarity of this species, which does not liquefy gelatin, is that the individuals of a colony are held together by such an extraordinarily viscid slime that even when they are allowed to lie for a week in water they do not separate. Hiltner suspects that they are able to fix nitrogen, but there is no possibility of confusing them with the true nodule bacteria. However, there are, as Beyerinck observed, several species of bacteria resembling the true nodule bacteria in the appearance of their colonies and in their general behavior which can give much trouble in the securing of pure cultures because of their frequent appearance in the nodules. Only a test of the nodule producing ability of a culture can here prevent mistakes. Additional certainty is obtained by determining under what circumstances bacteroids are formed.

But even when it is known that a culture consists of the true nodule bacteria, one does not know with any certainty whether he has the desired adaptation form. It seems indeed at first sight as if it would be sufficient to know the immediate source of the cultures. Any one who has not

studied this question carefully would, for example, take for granted that a colony of true nodule bacteria taken from a sound pea nodule would be composed of pea bacteria. Hiltner, however, from many observations and much reflection has learned to be very cautious on this point. He asks, for instance, whether he has any guarantee that he is obtaining true pea bacteria when he uses for obtaining his pure cultures pea nodules from Dahlem soil. In the Dahlem soil peas, vetches, clovers, lupins and serradella produce root-nodules without inoculations. Therefore, might not clover or lupin bacteria have wandered in after the way had been prepared by the true pea bacteria, and in consequence, might not the colonies chosen for further cultivation consist of clover bacteria instead of pea bacteria. To avoid this possibility, cultures for inoculation should not be set from single colonies. If the nodule bacteria comprise only one widely adapted species, as Nobbe and Hiltner formerly believed, it might be assumed that even these stray forms would by growth in the pea attain a greater or less degree of adaptation so that they could produce nodules on this plant. This conclusion is correct so long as the hypothesis holds true, but this is the case only to a limited degree. More recent experiments have led Hiltner to discard the single species theory and to distinguish two sharply defined groups which have the character of distinct species: One group contains *Pisum*, *Vicia*, *Lathyrus*, *Phaseolus*, *Trifolium*, *Medicago*, *Anthyllis*, *Onobrychis*, and *Robinia*; the other contains *Lupinus*, *Ornithopus*, *Soja*, *Genista*(?) and *Sarothamnus*(?). Buhlert in reaching his conclusion that but one species exists used only bacteria from the first of these groups, i. e., from *Vicia faba* and *Pisum sativum*.

Hiltner states that at no time has he considered a thoroughly adapted form as an unalterable one.

Mazé rejected the adaptation theory and distinguished two groups based on the acidity or alkalinity of the soil in which they live. Hiltner's experiments on this subject make such a view untenable, e. g., the *Robinia* bacteria do not cause tubercles on pea roots or vice versa, and yet the *Robinia* is not injured by lime. In the experimental garden at Hohenheim, of 13 kinds of lupins growing close together, 11 bore root-nodules and 2 were free. The soy-bean bacteria certainly belong with the lupin bacteria in morphology and biological peculiarities, yet the soy is a kind of bean and not hostile to lime.

Mazé's view that nitrogen assimilation takes place whenever an organism is able to cause nodules on a plant is also incorrect; for example, pea bacteria may penetrate into bean roots forming hundreds of nodules, yet often no nitrogen assimilation takes place.

His assumption that inoculation is of no use in soil containing no nodule bacteria since this fact of itself shows that they cannot grow there is unjustified because Hiltner's first work sufficed to show the contrary. Moreover, a careful study of literature would have shown both Mazé and Stoklasa that pure cultures of the pea bacteria were obtained direct from the soil at Tharandt as long ago as 1890.

In general, Mazé's theories are of this character that in their expression he disregards the results accumulated in abundance by different investigators during 10 years. Concerning the strange pleomorphisms reported by Mazé, Hiltner says comment is unnecessary.

As a nutrient medium, Hiltner has used Beyerinck's medium, viz., gelatin with extract of leguminous leaves, 0.5 per cent cane-sugar, 0.25 per cent asparagin and a little malic acid, but has found it advantageous to substitute root-extract for leaf-extract. After evaporating and drying the extract at 102° C. so that definite amounts might be taken each time, a 0.2 per cent solution was made to which was added 1 per cent grape-sugar and 0.1 per cent to 0.2 per cent asparagin. Only the best gelatin, e. g., Gribler's, must be used. Very acid gelatin should be avoided since its neutralization with sodium or potassium hydroxide introduces too much chloride. After the first cooking, the gelatin is neutralized with soda or potash and made moderately acid (deutlich) to litmus paper with malic acid. A second good medium for nodule bacteria is agar containing 2 per cent legume extract, 1 per cent grape-sugar, and 0.1 to 0.2 per cent asparagin. The root-extract gives enough acidity. No alkalis should be added, nor malic acid, since excess of acid in agar interferes with growth, which is not the case in the gelatin. Both the gelatin and agar should be heated as little as possible.

All the forms prosper on the agar but not all on the gelatin as already mentioned, i. e., lupin, serradella, and soja do not. The gelatin was not rendered suitable to them by omission of the asparagin, or by the addition of 10, 20, or 30 cc. of normal soda solution, or by the same amount of normal malic acid solution, or by addition of CaCO<sub>3</sub> after acidifying with malic acid. The addition of asparagin had no good effect. For these bacteria agar was found the most suitable medium when neither acid nor alkaline, but made either neutral or nearly neutral to phenolphthalein, with CaCO<sub>3</sub>. The following composition gave the best results: 1.5 per cent agar, 2 per cent root-extract, 1 per cent grape-sugar, heated in the autoclave 20 minutes at 120°. To 0.5 liter of this solution is added a knife point full of carbonate of lime. The mixture is then heated 10 minutes at 120° and filtered.

Tests with the addition of different amounts of pepton gave varying results. Soja bacteria were not injured even by 10 per cent pepton, while when 1 per cent pepton was present in the agar, pea

bacteria formed colonies with watery exudations and made no growth at all in higher concentrations (5 per cent). On the other hand, the addition of 0.3 per cent gelatin prevented the growth of Soja bacteria (in *Faba bouillon*) while it did not influence the growth of pea bacteria.

The very definite difference between the two groups shown by their behavior on gelatin is also a ground for the establishment of two species.

These results explain why it has been so difficult to obtain cultures from lupin or serradella on gelatin. When luxuriant colonies have appeared they were certainly formed not by *Rh. Beyerinckii* but by *Rhizobium radicola* which had entered the nodule by chance. The infection of peas with bacteria obtained from lupin nodules in 1890 at Tharandt, therefore, gives evidence that pea bacteria may be contained in lupin nodules, for the true lupin bacteria are not able to cause nodules on peas.

Experiments in pots were made with soy-bean plants using for inoculation pure cultures and also the contents taken directly from nodules. The latter gave the better results, though improvement in growth began 3 days earlier with pure cultures. In field experiments with the same inoculating materials the nodules being rubbed up and added to pure water until it was twice as cloudy as the pure culture suspension, the pure cultures produced very materially better results than the nodule contents.

In an experiment already referred to in which, after soil inoculation with soy-bean bacteria, oats were cultivated the first year and soja the second year, the soy-bean plants were almost free from nodules. In other tests with soy-bean in which the inoculations were made with bacteria mixed with either quartz sand, forest humus, compost earth, Dahlem earth, or water, only the quartz sand inoculation gave satisfactory results. Since it is not likely that the sand increased the multiplication of the bacteria the various soils must have reduced the number of bacteria within a very short time. Plants inoculated with compost earth formed on the two plots an average of 0.32 and 1.89 nodules per plant, those with Dahlem soil 0.12 and 1.00 nodules per plant, while those with quartz sand formed an average of 7.07 and 15.33 nodules per plant. Sterilizing the earths did not improve them. When Dahlem soil was mixed with quicklime (1 kg. earth and 2 gr. lime intimately mixed) and then with bacteria for inoculating material, no nodule formation occurred. These results do not hold for all limed earth, however, only in the case of quicklime. This was still able to act injuriously after lying in moist earth 24 hours before the bacteria were added. Had a longer time elapsed before the addition of the bacteria probably they would not have been injured. From this it is evident that unless one is dealing with acid soil, quicklime should not be used immediately before inoculation. Experiments in pots made at the same time and under the same conditions gave the same results, thus confirming Salfeld's conclusion that caustic lime exerts an injurious influence on the nodule bacteria.

Long before Hartleb brought out his specific nutrient fluid, the relative advantages of solid and liquid media were tested by Nobbe and Hiltner who found that the liquid medium offered most advantage because the bacteria remained much longer alive and capable of causing infection. Although these researches were not published, Hiltner has taken this fact into account by keeping all his cultures, which are to be further cultivated, in liquid media. Over against this undeniable advantage must be set the fact that a liquid culture medium does not permit the necessary control of the purity of cultures. Hartleb's claim that only bacteroids in liquid media were virulent while the bacteria on solid media were completely inactive, shows that he was not sufficiently familiar with the methods of cultivation on solid media. Certainly his statement is not of general application.

The previously noted experiments of Hiltner with the nodule contents in contrast to pure cultures gave evidence that the inoculating material loses in value as the bacteroids develop further from the original bacterial form.

The addition of saltpeter to grape-sugar solutions in which pure cultures were grown strongly suppressed the nodule producing ability of the bacteria. While pure cultures in 1 per cent grape-sugar solution produced when used for inoculation an average of 19.2 nodules per plant such a solution with 0.1 per cent saltpeter produced an average of 8.5 nodules.

The word *virulence*, says Hiltner, is a term used to express the degree of ability of the nodule bacteria to penetrate into the root tissues of the plants and to multiply therein. It is almost self-evident that the conditions of nutrition influence materially the degree of virulence.

In a series of experiments upon the spread of nodule bacteria in forest soil it was found that locusts grown in the middle of an old beech forest formed when uninoculated only a few small nodules and developed very weakly. When inoculated the growth from the beginning was luxuriant and the nodules were numerous. The results on the uninoculated plants led Hiltner to conclude that nodule bacteria may sometimes exist an extraordinarily long time in the soil without leguminous plants and that to all appearances, they are not dependent on symbiosis. It was noticeable that the nodules of inoculated plants were more active than those arising from bacteria already in the soil. This is referable rather to a difference in virulence than to a difference in the quantity of bacteria concerned.

This is not proved to a certainty, however, by these experiments. More convincing were the

experiments on high moorland at Bremen. In this case the strikingly large nodules formed on the yellow lupin from spontaneous infection were completely inactive, because of the poor degree of virulence [word here used with wider meaning] of the invading bacteria.

Experiments with soy-beans in pots confirmed this result and also explains how this increase in virulence can be brought about. Pots of Dahlem earth and sand, half of which were inoculated with Japanese soy-bean earth, were planted with soy-beans. Only late in the season did the difference appear between the inoculated and uninoculated plants. The greatest difference, however, was observable at maturity. The leaves of the uninoculated plants were then almost white, having lost almost completely their xanthophyll and their roots were free from nodules while the leaves of the inoculated plants were deep yellow and their lateral roots full of nodules as large as peas. The amount of dry substance from the inoculated plants was 33 per cent greater, and of seed about 74 per cent greater than that from the uninoculated plants. The next year plants in these same pots of soil without further inoculation showed an even greater difference. Those in the uninoculated soil remained completely nodule-free while the others showed extraordinarily strong nodule formation, so that every root on the upper third was closely covered with nodules the size of peas. Moreover the activity of the nodules the second year was remarkably increased. Even if it is very probable that the increased number of bacteria in the soil plays a rôle here, and that because of the great lack of nitrogen in the soil the nodules became active earlier and hence had a longer season in which to work, nevertheless the most important factor must be sought in the increased virulence, for while the total amount of nodule formation was considerably greater the second year it was not so much so as to go parallel with the increase in activity. Increased virulence here, as in organisms pathogenic to animals, is due to repeated passage through the organism in question. The dry substance of the uninoculated the first year being taken as 100, we have the following yields:

Year.	Uninoculated.	Inoculated.
1900	100	131
1901	66.4	145.8

Hiltner took this into consideration in obtaining virulent cultures for inoculation for all important species of legumes, and with few exceptions his cultures were passed several times through the appropriate host-plant. The better results of 1901 and 1902 in the open field he thinks is largely referable to this fact.

The chain of evidence for the greater activity of virulent bacteria was completed by making simultaneous inoculation experiments with bacteria of differing virulence, mostly on peas. The results entirely confirmed the preceding observations.

The effect of difference in virulence is also shown in the case where soil from Zehringer in Anhalt in which peas were known to form active nodules only on the deeper roots was used for inoculation in comparison with a third generation of pure cultures. The result was that the bacteria from the pure cultures penetrated the roots at once and formed numerous nodules on all the upper parts of the roots while those from the Zehringer earth produced nodules only on the deeper side roots and sparingly at that, *i. e.*, as in the fields. Only virulence can explain why the bacteria in the Zehringer soil did not penetrate the roots at once. Hiltner thinks that a moderate degree of virulence on the part of the bacteria is sufficient to cause leguminous plants to attain their maximum size. He thinks it also unquestionable that virulence may be increased in other ways than by repeated passage through the plant, *i. e.*, as Remy showed by feeding the inoculated plant with saltpeter.

It is an important question for agriculture whether one can not obtain too great a degree of virulence. It has been shown that at first the bacteria act toward the plant as real parasites. If this is correct, as appears on more grounds than one, we have to reckon with the possibility that the fight will be the more severe for the plant as the bacteria become more virulent. Indeed Nobbe and Hiltner record an experiment in which highly virulent pea bacteria caused direct injury to the plants growing in pots of nitrogen-free sand. In this case the bacteria penetrated the roots in such numbers that almost every root-hair was infected, and everywhere numerous nodules appeared in which, however, bacteroid formation was suppressed owing to the excessive growth of the bacteria. As a result no nitrogen assimilation occurred and the aerial parts of the plants were plainly weakened. Another experiment in 1902 at Dahlem on pot plants with especially virulent bacteria resulted in a decrease in the crop: Inoculated plants gave a dry substance amounting to 34.1 g. per pot.\* while uninoculated plants gave 47.1 g.

\*Possibly in some of these cases *Bact. tumefaciens* may have been the cause of the nodules. See Crown-gall: Its cause and remedy. Bull. 213, B. P. Ind., U. S. Dept. Agric., 1911, plate xvi, 2a.

On account of these undesirable results Hiltner was inclined to give up the use of such virulent cultures for practical agriculture and was deterred from doing so only by the idea that the better nourished plants in the open field would better withstand the bacteria than would potted plants. The results of field inoculations in 1902 justified this opinion. There might, however, be field conditions in which injury would result from very virulent cultures.

The benefit to the plant does not take place by absorption of the bacteroids. In the case recorded where absorption occurred there was no observable increased growth of the lupins.

It is desirable, therefore, to obtain bacteria with a degree of virulence sufficient to cause a strong root-infection yet not strong enough to cause injury. The best method of obtaining such cultures is as yet theoretical. Hiltner considers the solution of the problem to be possible by keeping the virulent bacteria on media as rich as possible in carbohydrates for some time before using them.

It is evident at any rate that nitrogen assimilation and virulence do not always run parallel and that the solution of the problem here presented promises better results in the use of pure cultures.

In 1902 a new method of seed inoculation was tried in which, by the addition of milk instead of water, the injurious action of the substance in the seed-coat was suppressed. The idea originated with Spiegel who obtained good results with clover in this way.

Good results with lupin were obtained on the birch moor at Bremen not only with milk but with a 3 per cent pepton solution and also with a solution containing 2 per cent grape-sugar and 3 per cent pepton, to spread the bacteria on the seeds. When water was used in the direct inoculation of seeds complete failure resulted. The use of milk gave such good results with lupin that it seems desirable to experiment with it further.

It remains to be proved how these means would act in other soils. The evidence seems sufficient, however, to warrant the belief that the injurious action of the seed-coat substance may be overcome without a preliminary swelling or germination of the seeds.

For the present it is recommended that for large seeded varieties the soaking of the seeds be avoided. Instead, they should be mixed with a corresponding amount of limed moor soil to which the bacteria have been added and which has been adapted to this use by the addition of grape-sugar and pepton. For small seeded sorts, such as clover and serradella, however, the methods of direct seed inoculation which have already proved successful are recommended.

Dr. Moore's bulletin on soil inoculation for legumes was published in 1905 after several years of experimental work. It opens with a summary of previous literature and closes with abstracts from reports of farmers favorable or otherwise. From November 1902, to November 1904, the U. S. Department of Agriculture sent out 12,500 packages of inoculating material. This inoculating material was dried on cotton. With the cotton were transmitted two small packages of nutrient substances with directions for use in preparing the cultures: One package contained sugar, magnesium sulphate, and potassium phosphate; the other ammonium phosphate. The contents of the first package was to be put into one gallon of clean water along with the cotton holding the dried bacteria, the ammonium phosphate was to be added 24 hours later and the fluid held till it became well clouded.

The bacteria were plated out and grown on 1 per cent agar containing 1 per cent maltose, 0.1 monopotassium phosphate, and 0.02 mag. sulphate in 100 cc. distilled water, and are said to become much more virulent on this medium than on that containing peptone or other nitrogenous substances. Field experiments by the acre are said to have demonstrated the much greater nodule producing power of the organisms grown on the non-nitrogenous media.

"As the result of numerous trials, however [which are not described], it has been found that although the bacteria increase most rapidly upon a medium rich in nitrogen, the resulting growth is usually of very much reduced virulence, and when put into the soil these organisms have lost the ability to break up into the minute forms necessary to penetrate the root hairs. They likewise lose the power of fixing atmospheric nitrogen."

The optimum temperature for growth is 23° to 25° C. The maximum 40° C. The minimum 10° C., "below 10° C. practically no multiplication took place." Air is necessary. Cultures in sealed tubes deteriorated rapidly. The bacteria in the soil will stand any degree of acidity or alkalinity that is not prejudicial to the host-plants. Potassium and sodium salts tend to inhibit the formation of the bacteria. Calcium and magnesium salts greatly favor their production.

"Alkaline nitrates in the proportion of 1 to 10,000 are sufficient to prevent the formation of nodules. \* \* \* the cultivation of the bacteria upon media containing appreciable quantities of nitrogen for any length of time is sufficient to cause them to lose both the power of infection and that of fixing atmospheric nitrogen."

Following Peirce the organism is regarded as a parasite, the doctrine of symbiosis being discredited. The host-plant is supposed to obtain its nitrogen by absorbing the bacteria. Ideas similar to Hiltner's are expressed, *e. g.*, that potassium nitrate in cultures reduces their power to produce nodules, that plants may bear inactive nodules and that "there is every reason to believe that when



land contains bacteria of a less degree of virulence than those sent out in the Department cultures an inoculation is worth while."

The organism is named *Pseudomonas radicola* (Beyr.) Moore. Sometimes it may be present abundantly in roots which show no nodules, *e. g.*, in berseem and in alfalfa. Suspected also to have been present once in this way in soy-bean and once in white lupin.

At Dr. Moore's request the chemists of the U. S. Department of Agriculture made nitrogen determinations from many of his flask cultures. These experiments are not given in detail but only referred to in a general way. The culture medium consisted of mag. sulphate, potassium phosphate, and maltose in water, and preliminary determinations showed per 100 cc. only 0.0003 gram of nitrogen (as nitrite). After inoculation, air was drawn through the flasks (90 in all) by an aspirator, after first passing through a flask filled with pumice stone and sulphuric acid.

The actual gain in nitrogen in the inoculated flasks at the end of the third week varied from 0.0002 gram to 0.0022 gram per 100 cc.

"The checks or uninoculated flasks of which there were twelve, four being analyzed at the end of each week, at no time showed an increase over the original 0.0003 gram per 100 cc."

It is not stated whether these also were aspirated.

A second series of flasks was started some weeks later to determine whether the increased nitrogen was combined with potassium in the fluid or was contained in the cells of the bacteria. After some weeks' growth the filtrate and fluid were analyzed separately, the results showing that most of the nitrogen was held in the bacteria themselves.

It is also stated that "Analyses of the nodules of legumes show that they frequently contain as high as 7 to 8 per cent of nitrogen, while other parts of the plant will not possess more than 2 per cent." It is not stated *what other parts* were compared, *i. e.*, whether equally young parts which would make a very great difference.

"The large rods will withstand desiccation for a year or more, and, therefore, because they may be sent dry any distance and upon being revived be in the same condition of efficiency with which they started, the problem becomes a very simple one."

The probability, however, is that the problem is much more complex than here supposed, although the writer of this book must continue to believe that many of the cultures sent out on cotton were virulent.

Owing to criticism of various workers at home and abroad who declared the method to be worthless, the Department of Agriculture soon abandoned distribution of the organism on cotton, and now sends out its pure cultures sealed in glass tubes suspended in fluid.

In this connection, however, see favorable comment under Peglion (1905) in Literature. Of the 2,502 reports received by Dr. Moore from growers 1,296 reported an increase in crop on the inoculated part of the field, or on the whole field as compared with previous years, and not a few of the reports were extremely favorable, the following for example:

"On 53 untreated vines [peas], taken as they came, I found 102 pods; on 53 treated vines taken as they came in the next row, I found 856 pods. The first picking well-nigh stripped the untreated row; the treated ones have yielded two good pickings since, and still another is now filling out."

Kruijff's criticism (1907) is as follows: The root-nodule bacteria of leguminous plants will not withstand drying upon cotton for any great length of time, and this method introduced by Moore is worthless. The fluid prepared in the manner directed by him, *i. e.*, with the packages of ammonium phosphate and sugar, and inoculated with the cotton received from America clouded, it is true, but a microscopic examination showed that the micro-organisms in it were mostly other than the nodule organism. Once it consisted almost entirely of yeasts. Streaks on agar made from distilled water in which the cotton had been soaked gave only rare colonies of the organism. The appearance of the other organisms is attributed to careless preparation of the cotton. Because it was believed that the virulence of the organism on the cotton from America had been increased by its method of cultivation, they were very anxious at Buitenzorg to try it. Inoculations were, therefore, made from the colonies obtained on the agar streaks, but with no favorable result. In two cases out of six an increased yield was obtained but with bacteria isolated in Java. The increase in one case was 15 per cent (grown on Hiltner's media) and in the other 17 per cent (grown on Moore's nitrogen poor agar). Soy-beans were used for the first mentioned experiment and Katjang tjina (peanut?) for the second.

Harrison and Barlow in Canada have recently published on this subject (1907).

They examined the roots of 30 foreign economic species of the suborder Papilionaceae, 24 species and varieties of *Vicia*, etc. Nodules were found on the roots of all with the exception of *Cicer arietinum* and *Galega officinalis*. In the suborder Caesalpiniaceae they examined the roots of *Gymnocladus*, *Gleditschia*, *Cercis canadensis* and no nodules were found, mycorrhiza, however, were present in all cases.

They isolated an organism believed to be the cause of the nodules from the following species:

Trifoliaceae: *Medicago sativa*, *Melilotus alba*, *Trifolium incarnatum*, *T. pratense*, *T. repens*.

Hedysaraceae: *Desmodium acuminatum*, *D. canescens*, *D. nudiflorum*.

Viciaceae: *Vicia villosa*, *Lathyrus sativus*, *Pisum sativum*.

Phaseoleae: *Glycine hispida*, *Apios tuberosa*, *Phaseolus vulgaris*.

After experimenting with various media, they settled down to the use of a fluid medium containing water, wood ashes, and maltose. In case a solid medium was desired, agar was added and occasionally monopotassium phosphate. This medium is adapted both to the host-plant and to the bacterium. They state that *Bacterium leguminosarum* grows well in each one of the following liquid media:

- (1) 1,000 cc. distilled water; 15 grams wood ashes.
- (2) 400 cc. above solution filtered, with 4 grams of maltose.
- (3) 200 cc. of first solution with 100 cc. distilled water, and 3 grams of maltose.

These three solutions should be heated for half an hour in streaming steam and then boiled a moment over an open flame, or heated for 10 minutes at 10 pounds pressure in the autoclave. The fluids are then filtered clear, put into tubes and sterilized in streaming steam or by exposure in the autoclave for 10 to 20 minutes at 10 pounds pressure. The agar media is prepared in the same general way as the above with the addition of agar. They state that when 1,000 cc. of water is used the sugar may vary from 4 to 20 parts; the wood ashes from 0.0 to 50 parts; the agar from 7.5 to 15 parts; and the acid potassium phosphate, when this is used, from 5 to 10 parts. The paper should be consulted for details concerning the preparation and use of the media.

They state that the ash-maltose-agar in Erlenmeyer flasks has many advantages over quartz or water culture: one of which is that the agar contains only about 1 per cent of inert material, whereas the quartz contains about 60 per cent; another is that the root-hairs and the forming nodules can be seen in all parts of the agar but only when next the glass in case of quartz-sand cultures.

"Growing the plant within a glass flask affords several advantages and offers few technical difficulties. It makes possible the most rigid pure culture methods; it requires no attention beyond the initial preparation; that is, the medium does not require to be restored nor renewed, even during a period of growth of eight months."

The organism was isolated from the nodule in customary ways, the surface being sterilized after washing by immersion in about 20 cc. of 1:500, or 1:1,000 mercuric chloride water, to which 2.5 cc. of c. p. hydrochloric acid (sp. gr. 1.20) had been added. In case the nodule floats it must be held under by means of a glass rod. If it is small it may remain immersed 2 to 3 minutes, but not more than 5 minutes. Large nodules may remain in the solution for half an hour. The colonies vary with the plant from which the cultures are made, also with the conditions of the media, and are characteristic. The deep colonies are circular, elliptical, and triangular with rounded corners. When they rise to the surface they take on the form of surface colonies, except that at the center they show their submerged origin. Deep colonies do not grow as well as surface colonies. They are granular, white by reflected light and brownish with transmitted light. The surface colonies are raised, round, wet, entire, shining and white. They appear like drops of melted paraffin. At first they are gleaming and transparent, then translucent, then gradually more turbid and finally opaque. At first they are watery, but they may finally become very viscid. The surface colonies may attain a diameter of 1 to 2 mm. in 5 days and 3 to 4 mm. in 15 days.

They state that they have failed to detect the presence of any other organism in leguminous nodules. When the interior of a nodule was inoculated into media and plates were poured, either the plates remained sterile or else *Bacterium leguminosarum* developed. Occasionally, however, mold colonies appeared and other extraneous bacteria. In stab-cultures in the ash-maltose-agar, after 2 or 3 days at 25° C., there was a raised, circular, transparent, wet-shining growth, spreading on the surface from the point of inoculation, and a filiform growth along the needle-track which sometimes had fine filaments radiating from it horizontally into the agar. These filaments were shorter toward the bottom of the stab. Cultures on the ash-maltose-agar in Freudenreich flasks remain alive for more than a year at room-temperature.

In ash-maltose-water media the liquid becomes turbid and a sediment forms which is not ropy but diffuses on shaking and there appears on the glass a thin wide ring of growth from the surface of the fluid downward. Media consisting of distilled water 100 parts, maltose\* 1 part, and ashes 0.5, 1 and 1.5 parts, and varying from neutral to -3°, is favorable to the growth, which begins usually in 3 or 4 days and increases visibly for 15 days, the liquid becoming turbid and the turbidity continuing with the formation of a thick white layer or precipitate. When the ashes were increased to 2 or 2.5 parts per 100, there was a less satisfactory growth, the body of the liquid remaining clear and the slimy white growth taking place in the bottom of the tubes in 15 days or more.

\*The maltose used by Harrison and Barlow, was probably not pure. According to Mr. Barlow it was yellowish.

The morphology of the bacteria varied with the species of legume, the age and size of the nodule, the portion of the nodule examined, and the conditions of infection and growth. In plants of the tribe Phaseoleae the bacteria were mostly small rods with comparatively few branching and irregular cells. In plants of the tribe Trifolieae branching and irregular forms prevailed. In general, although not always, simple rods prevailed in young and small nodules and branched irregular forms in older and larger nodules. The proximal part of the nodule, that part first formed, may contain simple rods mainly, and the distal part, where growth is taking place rapidly, may contain not only simple rods but many branched or twice branched forms.

The authors state that they were able to see the polar flagellum of this organism unstained by taking a loop of the mucilaginous or viscid growth of an agar culture a few days to several months old and spreading it out in streaks on a clean slide, lashing it out into slender tongues. The film was allowed to dry in the air but not killed or fixed in any way. It was then flooded for a moment with a saturated alcoholic solution of gentian violet to stain the mucilage, washed under the tap, dried between folds of filter paper, and examined with an oil immersion lens. The mucilage stained deeply, and the flagella not at all. On the margins of this slime they frequently found the flagellum visible as a long colorless thread attached to the pole of the bacterium.\* Kiskalt's amyli-Gram stain is stated to be very good for staining *Bacterium leguminosarum*, especially from the nodules, since the amyli alcohol clears up the background, without removing the stain from the bacteria, and also shows clearly the internal structure of the bacteria. The authors give a table, which includes the length of life of *Bacterium leguminosarum* from various legumes (white clover, red clover, alfalfa, vetch, flat pea, common pea, bean, *Desmodium*, and *Apios*). They found this to vary from 1 to 2 years when cultivated on solid media containing wood ash, maltose, acid phosphate, and agar. In most instances the organism lived more than 18 months, once it lived 2 years lacking 5 days; in 2 cases only was it dead at the end of 10 months.

The authors tried various methods of sterilizing the surface of seeds, viz., dry heat, moist heat, sulphuric acid, calcium hydrate, formalin, and mercuric chloride, but only in a few instances were living seeds obtained free from living bacteria, and never when the seeds were inoculated with the spores of bacteria. Either the seeds refused to germinate after treatment or else after germinating they were found to be still infected with bacteria. The authors finally obtained sterile seeds by another method, viz., by selecting well-developed, unopened pods, soaking these for an hour or more in 1:1,000 mercuric chloride water, and then placing them in folds of sterile cotton. The pods were also held in forceps, and passed through the flame on all sides and the ends were well burned. They were then opened, the seeds removed and placed between folds of sterile cotton. In a few days, when the seeds were dry, they were taken in flamed forceps and put into plugged sterile test tubes where they remained at room temperature until they were needed. In this connection see fig. 2.

To germinate the seeds, they were thrown into about 3 cc. of boiling distilled water in test tubes. The tubes were immediately cooled and incubated at 37° C. and then at 25° C., until the seeds germinated. After the first 24 hours the tubes were inclined so that the seeds were only partially under water. The germinated seeds from those tubes in which the water remained free from bacterial growth were then transferred to flasks containing the ash-maltose-agar, where the growth of the seedlings continued.

At first all of the plants grew vigorously on the agar, but in those flasks held as checks they began to dwindle after a time and either died outright or made a very unsatisfactory growth; whereas in those flasks which were inoculated with *Bacterium leguminosarum* the growth continued until the end of the experiments. One series of flasks was under observation for 8 months and three other series for shorter periods. The root-nodules began to appear in about 1 month. Sometimes they were few and large; at other times many and small. In one instance 70 developed on the roots of one plant. In the absence of root-hairs the infection began as a small transparent spot in the root. Nodule formation and general infection of the root appeared to check extensive root formation. *Bacterium leguminosarum* grew copiously on the surface of this agar and penetrated the agar wherever the roots penetrated it. A general infection of the roots usually accompanied the nodule formation. In flasks with ash-maltose-agar, nodules were obtained upon *Phaseolus vulgaris*, *Vicia villosa*, *Pisum sativum*, and *Glycine hispida*. Cross-inoculations were not observed; for instance, when pea and vetch were grown in the same flask and inoculated from a vetch culture, nodules formed only on the roots of the vetch. In the same way, when a bean and pea were grown in the same flask, inoculated from the pea culture, nodules formed only on the roots of the pea.

One can not tell from this paper whether or not the organisms used for inoculations were subcultured from colonies sufficiently to overcome de Rossi's objection. Barlow with whom I conversed thinks some of them were.

\*The writer has examined a slide of this kind prepared by Mr. Barlow. Only here and there does a rod show this phenomenon, most do not.

In 1907, Gino de Rossi contributed a paper on "The Micro-organism Which Causes the Root-nodules of the Leguminosae," taking a view quite different from the ordinary one.

He used a large number of plants of *Vicia faba* (fully 60). From each plant, except the youngest which had no nodules, 12 or 15 nodules were removed from different parts of the roots, and repeatedly washed in tap-water.

They were then placed in a stoppered vessel of sterilized distilled water. The water was changed twenty times in an hour, and each time the vessel was shaken vigorously. Finally, without other attempt at surface sterilization the nodules were placed by means of sterile forceps in a Petri-dish containing a few pieces of filter paper, the whole having been previously sterilized in the dry oven.

All liquids, instruments, and vessels used in the further handling of the nodules were very carefully sterilized by heat.

After every trace of water was removed by the filter paper, the nodules were removed to another Petri-dish where they were sectioned, and a small portion of the interior removed with a sharp needle, care being used not to touch the surface layers. This fragment was crushed out in 2 to 3 cc. of water in a stoppered vessel. Three or four such vessels were prepared from each plant. Microscopic preparations and cultures were made from this material.

The following solid culture-media were used:

- (a) Simple 10 per cent gelatin, with beef-extract (1 per cent), pepton (1 per cent) and sodium chloride (0.5 per cent), the reaction of which was mildly alkaline or mildly acid (natural acidity).
- (b) Ten per cent gelatin prepared with *Vicia faba* extract (1 part leaves cooked in 8 to 10 parts of tap-water and filtered), 1 to 2 per cent cane-sugar or glucose, 1 per cent pepton and 0.5 per cent sodium chloride, with a mildly acid or alkaline reaction.
- (c) Simple agar (1.5 per cent) or agar with *Vicia faba* extract. Poured plates were made. In some experiments all these media were used, in some only one. In every case, however, the somewhat acid gelatin containing *Vicia faba* extract was used.

De Rossi examined the contents of the nodules in hanging drops and in stained preparations. He found the contents of very young nodules to consist of non-motile rodlets, constant in breadth but not in length, and easily stained with aniline dyes. He did not find motile bacilli, either large or small.

In older stages of the nodules a series of transitions was observed, the rodlets showing first one end slightly swollen, then a slight dichotomy at the end, and finally the typical X and Y shapes, which, contrary to current statements, were constant in form and dimensions.

In this phase the central part of the nodule contains an enormous number of the branched forms; only in a few cases were straight rods seen. De Rossi considers these bacteroids as a stage in the development of the organism, not as degenerate forms.

He observed the development of what appeared to be vacuoles in the bacteroids, and states that this is a constant feature, belonging to a phase in the development of the organism. The bacteroids at this stage are somewhat swollen.

In no case did he see any very small infecting bacilli or the development of such bacilli into bacteroids.

Cultures, made on the solid media by flooding the surface with a dilution from nodules full of bacteroids, developed a small number of tiny, whitish colonies. These appeared in 2 or 3 days, at a temperature of 16° C. As development on the plates proceeded a mixture of very different colonies was often apparent.

By selecting plates with one dominant form, pure cultures of four sorts of Schizomycetes were made. This variety of forms together with the presence of numerous non-germinating bacteroids in the surface of the gelatin (as determined by a microscopic examination) suggested to de Rossi that the bacteroids were unable to grow on artificial media, and that the colonies present belonged to other organisms which had penetrated the nodule and multiplied there sparingly [they may have come from the surface], but which had nothing to do with its production. He further supposes that these non-infectious intruders have been commonly mistaken for the root-nodule organism. One white form was non-liquefying and motile by means of a polar flagellum.

To prove these hypotheses he isolated the bacteroids from the surface of such cultures in the following way:

After 12 to 15 days in the thermostat (probably at 15° to 20° C.), the surface of plates, which had yielded a few colonies only, was moistened, and scrapings from the apparently sterile parts between colonies were used for inoculating various media. The actual presence of numerous bacteroids on this surface was demonstrated by microscopic examination: The results were all negative. The media used were gelatin with mineral salts or *Vicia faba* extract, beet roots, and raw and cooked potato. Cultures were also attempted in pure nitrogen without positive result.

Inoculations were then made on plants of *Vicia faba* using: (1) Scrapings containing the bacteroids which would not grow on his media; (2) the fourth or fifth sub-cultures of the colonies which

grew readily, these distant sub-cultures being used so as to avoid carrying over any of the numerous bacteroids.

For planting, pots of washed and dried soil wrapped in paper were sterilized 1 hour in the autoclave at 134° C. Seeds of *Vicia faba* were soaked for half an hour in alcohol at 60° C., repeatedly washed in sterile distilled water and then dipped into one of the inoculating preparations. Inoculations were also made by adding 1 cc. of this inoculating material to the soil after sowing. Control pots for each experiment were prepared. No attempt was made to protect the soil from aerial contaminations, but as the control plants produced no nodules the author thinks such a precaution unnecessary. After 2.5 months growth, no trace of nodules appeared on the roots of the controls or of plants inoculated with subcultures from the colonies which grew readily on his plates, while those inoculated with the bacteroids had formed many nodules.

To meet the objection that failure to obtain nodules from his subcultures was due to loss of virulence, de Rossi states that loss of virulence commonly occurs in pathogenic bacteria only after a considerable time and many transfers, never after so short a period and so few transfers as in case of his organisms. Here loss of virulence is really loss of the invisible and hitherto unrecognized organism, *viz.*, the non-germinating bacteroids. What has commonly been considered to be the right organism is only an intruder.

In the course of further studies de Rossi discovered and isolated a very slow-growing organism which did not lose its virulence, and which he states to be quite unlike *Bacillus radicola* in its morphology and cultural characters, which are described as follows:

Young (non-vacuolated) bacteroids remain unchanged upon the plates (gelatin, agar, etc.) even when observed for 20 or 30 days or longer. As soon, however, as they become vacuolated they grow and form colonies. In this phase intruding colonies are fewer and often, for a time, the plates appear to be sterile. When, however, the plates are observed for a longer time, small formations are seen to appear (between the sixth and ninth days) which must be regarded as real colonies.

These colonies develop from the vacuolated bacteroids, a process which de Rossi states he watched under the microscope (compare with statements by Hiltner). The bacteroid lost its typical form, changing to an amorphous heap of more or less spherical, tiny, bodies. Multiplication of these went on, enlarging the colony which, however, remained microscopic. The colony was round and granular and composed of spherical, elongated, or bluntly branched bodies, held together by an apparently gelatinous substance. Photomicrographs of these are given.

From this stage on, the various nutrient media had different effects. Upon simple nutrient gelatin no further development took place; on gelatin with *Vicia faba* extract the colonies enlarged so as to be visible to the naked eye as minute points. This requires 12 to 30 days. These colonies were somewhat raised and non-liquefying. In three cases the spherical and branched forms occurring in the colonies were all transformed into rods. In eight other strains from as many plants this change did not occur or remained incomplete, roundish and irregularly branched forms being mixed in with rods, up to the close of the observations. Further observations convinced him that all of these were pure cultures.

Streak and stab cultures, in gelatin with *Vicia faba* extract, were made from colonies on these 8 plates. The development was extraordinarily slow; within 8 to 10 days a very minute growth was observed, which after 25 to 30 days had taken on a characteristic appearance. At the point of inoculation a tiny drop of colorless gelatin-like substance rose strongly above the surface of the gelatin, containing and surrounding a white stearine-like bacterial growth. This substance colored intensively in microscopic preparations, and showed that it surrounded the usual peculiar forms.

Four or five transfers in series were made and each time the growth was more luxuriant, the drops were whiter and the enveloping substance less abundant. At the same time the organism began to take on the form of rods. These rods stained with aniline dyes, but not by Gram's method. In fresh cultures diluted with water and watched in hanging drops many motile individuals were seen. De Rossi was unable to demonstrate the flagella. After 6 or 8 days the rods in these little colonies became vacuolated and, when placed on peptonized nutrient gelatin, developed just as the vacuolated bacteroids from the nodules had done, *i. e.*, they became swollen and granular and then changed into tiny heaps of more or less rounded bodies. On this medium no further development took place. When, however, these swollen granular bodies were placed on gelatin with *Vicia faba* extract, they multiplied by division, reproducing the bacterial form.

On other media, as bouillon, agar, potatoes, or carrots there was no development. In Moore's agar (containing little nitrogen), and in silicate jelly (no nitrogen) growth was good, forming a thin, white, wide-spreading layer.

Inoculations on *Vicia faba*, made as before, but with this newly isolated organism, gave most positive results.

It must be left an open question whether de Rossi's results are to be regarded as something entirely new and revolutionary or only as a record of certain difficulties which would not have been encountered by another investigator. In some ways, at least, the organism which he finally isolated is not unlike what has been considered in recent years by many bacteriologists as the root-nodule organism.

Regarding his inferences two or three countering ones may be made:

- (1) He entered the nodules through an unsterilized surface and, hence, the various sorts of colonies which grew promptly on his plates may have come from the surface and are not necessarily what others have mistaken for the root-nodule organism. Indeed, it is possible that all of these early difficulties might have been avoided by a proper technique of entrance.
- (2) His failures to obtain any growth of the right organism on ordinary nutrient gelatin are exactly what a careful study of the previous literature of the subject should have led him to expect. Beyerinck long ago divided the root-nodule organisms into two groups—those which grow well on ordinary media, *e. g.*, beef extract-peptone-gelatine, and those which do not, and stated that the organism from *Vicia faba* belonged to the second class.
- (3) The very slow growth of the right organism on gelatin containing *Vicia faba* extract may also have been due to the restraining influence of an improperly prepared culture medium, *i. e.*, one made from an inferior strongly acid gelatine, or one containing too much chloride, too much peptone, or too strong an extract of the green parts of the plant, or one boiled for too long a time.
- (4) Unless the experiments were duplicated several times with identical results we might assume that all of the bacteroids were dead in the one case (scrapings from the agar surface to other media) and only a part of them dead in the other case (scrapings from the agar surface to *Vicia* plants which became infected.)

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## BACTERIAL SYMBIOSIS IN OTHER GREEN PLANTS.

Hiltner evidently considers the root-swellings on *Alnus* as due to bacteria. He says bacteria are present in the nodules and that there is a symbiosis, or at least that *Alnus glutinosa* starves in nitrogen-free sand, or water, when they are absent and thrives in it when they are present. We must await his promised full paper on this subject. Meanwhile the reader is referred to his striking figure in Lafar's *Handbuch der Technischen Mykologie*, Bd. 3 (p. 63), where also may be found references to the literature of the subject. Similar claims are made for the Eleaginaceae, but the organism occurring in their root-nodules is not specified by him in any other way than that it resembles the one in *Alnus*, but is not the same. Very little is known respecting the nature of the root-nodules on *Melampyrum* and other genera referred to by De Vries, Beyerinck, etc. They are good subjects for further study.

A common root-symbiosis in Orchidaceae, Ericaceae, Cupuliferae, Coniferae and some other families is due to fungous filaments, the so-called *Mycorrhiza*, the literature of which is now considerable.

Some references to *Alnus*, etc., are given under Literature of Root-nodules of Leguminosae. Peklo says the growths are due to *Actinomyces*.

## INSECTIVOROUS PLANTS.

In 1889, Tischutkin, stimulated apparently by Morren's first paper, attacked the views of Charles Darwin and others respecting the insectivorous nature of *Pinguicula*, ascribing the solution of the albumen, etc., placed on the leaves, to the presence of bacteria.

He cultivated *Pinguicula* under bell jars. After stimulating the glands of the leaf to secretion by laying dead flies on the surface, he replaced the flies by little cubes of cooked egg albumen, and 18 to 22 hours later collected the leaves and put them into chemically pure glycerin. A few days later the glycerin extract was filtered. It gave an acid reaction as did the sap from the glands. He used this extract for seven series of experiments as follows:

In the first series he placed a piece of albumen and 2 cc. of distilled water in a test-tube. In the second and third, 2 cc. of dilute hydrochloric acid (0.2 per cent and 0.02 per cent respectively), replaced the distilled water. In the fourth, 5 drops of 0.2 hydrochloric acid were added to the formula of No. 1, and in the fifth, sixth, and seventh, 2 cc. of asoda solution (0.5 per cent, 0.25 per cent, and 0.05 per cent respectively), replaced the water in No. 1. To each of these was added the glycerin extract, beginning with 6 drops and gradually increasing it to 2 cc. In an eighth series gluten-fibrin or gelatin was used instead of albumen. All experiments were made at room temperatures. In all cases results were negative. There was no digestion of albumen or gelatin. The biuret reaction showed that no peptone was present. On starch also the extract had no effect.

As a control experiment, pieces of albumen were placed on the leaves and after 8 hours when excretion of fluid had taken place, these were allowed to remain in it or were replaced by fresh pieces of albumen, gelatin, or oxblood fibrin. Very small pieces of albumen were entirely dissolved, but larger pieces, even after 42 hours, were dissolved only in part, the remaining part becoming pulpy. The gelatin was always completely dissolved, small pieces in 24 hours, large ones after 40 to 42 hours. This part of his observations corresponds fully to Darwin's.

Considering it possible that some substance injurious to the ferment had been extracted by the glycerin, Tischutkin repeated these experiments, using, instead of a glycerin extract, the sap collected directly from the surface of the leaves by means of a capillary pipette. Part of this fluid was left in glycerin 4 or 5 days, the rest was diluted with a small quantity of distilled water. Again the result was negative. The addition of malic and formic acids did not alter the effect. Neither was there any digestion at a higher temperature (32° to 35° C.). For a control pure Russian pepsin was used with positive results.

Tischutkin thinks, therefore, that the solution of albumen on the leaves is due to some cause other than the action of a ferment secreted by the gland-cells. He concludes that the plant merely secretes a liquid favorable to the growth of bacteria, and that the bacteria produce the pepsin [trypsin] which dissolves the albumen.

This view is favored by the fact that a very considerable time is required to bring about this change (56 to 82 hours in case of fibrin) and also by the fact that gelatin, the most easily digested substance is known to be readily liquefied by a great number of micro-organisms.

In all, Tischutkin made over 150 experiments.

In 1892 Tischutkin published a second paper on carnivorous plants in which he attempted to show that micro-organisms are a constant feature in the fluid excreted by such plants, and that these organisms are able to dissolve albumen.

For his experiments, *Pinguicula vulgaris*, *Drosera longifolia*, *D. rotundifolia*, *Dionaea muscipula*, and *Nepenthes macleodiana* were used. The following is a synopsis of his paper.

Microscopic examination of the excreted sap 24 and 48 hours after stimulation, and also 3 to 5 days later, revealed the presence of bacteria and moulds. Myriads of bacteria were found in the extruded fluid of *Drosera* and *Dionaea* in 20 to 24 hours. A drop of the sap, 24 hours after stimulation of the glands, was placed by means of a flamed platinum loop, in sterile flesh-peptone-gelatin and after 3 dilutions in such gelatin Petri-dish poured plates were made from each of the 4 tubes. When colonies had developed, small portions of them were removed with a sterile platinum wire, placed in similar gelatin and kept at room temperature. After cultivation for 9 or 10 generations in this medium, test tubes of weakly acid, pepton-free, flesh bouillon and of weakly acid sterile water were inoculated from the cultures and a piece of albumen was added. The solution of albumen was rapid. Several species of such bacteria were found, one or more kinds on each insectivorous plant, 4 sorts on *Pinguicula*.

The sap from unopened but fully developed pitchers of *Nepenthes* was unable to dissolve albumen. A piece of albumen was inserted into two such pitchers and the opening carefully closed with animal plaster. One of these was *N. distillatoria*, the other *N. hirsuta*. In 4 days the pitchers opened. At this time the sap contained no pepton and extremely few bacteria. The albumen was unmodified. In Rothert's review of Tischutkin's Russian paper it is also stated that the sap had a strongly acid reaction. This same sap when poured into test tubes and kept at 20° to 21°, dissolved the albumen in 4 to 5 days but then the bacteria had multiplied enormously.

From these results he draws the conclusion that the solution of albumen is due, not to the secretion of the plant, but to the action of peptonizing bacteria living in it, and beginning only when these organisms have become very numerous. They are not present in the unopened pitchers.

The dry residue left by evaporation of pitcher liquid, according to Völker's analysis, varies from 0.92 per cent to 0.85 per cent, and is composed of: malic and a little citric acids, 38.61; potassium chloride, 50.42; carbonate of soda, 6.36; lime, 2.59; magnesia, 2.59; organic material, a trace.

The action of the extruded fluids has nothing in common with animal digestive fluids. We may regard the relation of the bacteria to these plants as a symbiosis in which the higher plant supplies the food for the bacteria and takes in return soluble animal products digested by the latter.

In 1897 Vines published a paper on the proteolytic enzyme of *Nepenthes*, in which he endeavored to confirm his earlier statements and to refute the views of Dubois and Tischutkin.

In his experiments he used, chiefly, as material for digestion, well-washed blood-fibrin which had been preserved in a mixture of one part pure glycerin and two parts distilled water. He says that such fibrin may be regarded as free from bacteria in view of the antiseptic action of glycerin (Copeman and Blaxall, Br. Asso. Rep., 1896). He never failed to obtain digestion of fibrin by the pitcher liquid when an adequate amount of acid (0.25 per cent solution of hydrochloric) was present. In control tubes of dilute hydrochloric acid and fibrin, the latter was softened in the time required for digestion with pitcher liquid, but never digested.

Experiments were made to determine whether this digestion would go on without the presence of bacteria.

"Three test tubes were prepared as follows: Each tube contained some pitcher liquid acidified with 0.25 per cent hydrochloric acid, and a shred of fibrin. To (1) was added some potassium cyanide; to (2) some thymol; to (3) a few drops of chloroform.\* At 1 P. M. the tubes were placed in the incubator at 35° C. The fibrin in tube (1) was completely dissolved by 5 P. M.; that in the other two tubes was broken up by that time and was found to have entirely dissolved by the following morning at 9 A. M."

Similar experiments using egg albumen and others using fibrin and corrosive sublimate gave satisfactory results (10 cc. pitcher liquid, 5 cc. 0.25 per cent hydrochloric acid, 5 cc. 1 per cent mercuric

\*Thymol and chloroform, it should be remembered do not restrain all bacteria, nor is potassium cyanide generally reckoned as a strong germicide.



chloride). Fibrin was dissolved in 4 hours or less. Hydrocyanic acid, which according to Fiechter (Basel, 1875) does not injure enzymes while it arrests the actions of, and even kills, yeasts and bacteria, was used with equally favorable results. Fibrin (50 mgs.) was digested in 1.5 hours in a solution made up as follows: 5 cc. pitcher liquid, 5 cc. of 2 per cent solution HCN, and one drop of strong hydrochloric acid.

These results seemed to exclude the hypothesis of bacterial action, yet Vines thought best to strengthen his position by experiments on the other side; *i. e.*, to use conditions favorable to bacterial growth, so said, but fatal to enzymes. To this end he treated pitcher liquid, at a temperature of 35° to 40° C., with alkalis in various strengths and for various periods, and after neutralizing it, tested its digestive activity. He found that the activity of the liquid was destroyed by treatment with 1 per cent sodium hydrate for 1 hour, and by treatment with 5 per cent sodium carbonate for 3 hours. These alkalis produced a precipitate in the pitcher liquid.

A study of the relation of digestive activity to the amount of free acid present in the liquid gave the following results: Tubes A, B, C, and D, containing respectively 1 per cent, 0.5 per cent, 0.25 per cent, and 0.125 per cent of hydrochloric acid, kept in the incubator, digested 1 gram of fibrin in the order of rapidity of C, B, A, D, that in D not disappearing within 2 hours while C required only half an hour.

The author succeeded in removing from the pitcher liquid a substance with which a new digestive liquid could be prepared. This he did by obtaining a bulky precipitate with absolute alcohol, phosphoric acid, and lime water, the liquid being finally neutralized with ammonium carbonate. This precipitate was shaken up with a 0.25 per cent solution of HCl, and the liquid filtered. In this liquid rapid digestion of fibrin took place with good biuret-reaction. A month later the same success was obtained by using some of the precipitate which had been kept dry in the presence of chloroform vapor.

The liquid of unopened pitchers was found to be generally acid, that of opened ones, either acid or neutral. The pitcher liquid is quite inert in the absence of acid. Only a minute quantity of proteid was present, nor was there evidence of the presence of zymogen. Hence he thinks that either the enzyme is not a proteid, or if it is, is present in extremely minute quantity, though it is difficult to accept the second view because of the remarkable digestive activity of the liquid.

Active glycerin extracts were prepared, some with, others without previous treatment of the pitcher-material with absolute alcohol. The extract from untreated material was the more active in the digestion of fibrin. These pure glycerin extracts did not retain their activity for a prolonged period but one was active at the end of 2 months. Only relatively young pitchers yielded this extract. This indicates that enzyme secretion ceases some time before the pitchers show signs of withering.

The presence of a digestive agent in the plant tissues affords collateral evidence that this agent is not a bacterium but an enzyme. Vines thinks it highly improbable that any bacterium would retain its vitality after two months sojourn in pure glycerin.\* His observations led him to differ with Gorup-Besanez who described the products of digestion as peptones. He finds the main product to be an albumose, allied to deuto-albumose, and failed to detect the presence of a true peptone. He says:

"The only certain proof that a liquid contains true peptones lies in the fact that such a liquid continues to give proteid reactions after saturation with ammonium sulphate, since this neutral salt precipitates all proteids except true peptones; but this test was not applied by Gorup-Besanez in any of the above-mentioned researches. As I have already pointed out, I have invariably found that a solution of the products of digestion by pitcher-liquid gives no proteid reaction after saturation with ammonium sulphate, all the proteids present having been precipitated by the salt."

No readily dialysable proteid was demonstrated. The nature of the ultimate product of digestion was not determined with complete success, though Vines thinks it may be leucin or some allied body.

He lays stress on the fact that the liquid of unopened pitchers of *N. mastersiana* is distinctly acid, a fact which controverts the idea that the secretion of acid is the result of stimulation.

In 1898 Vines published a paper on the proteolytic enzyme of *Nepenthes* in which he recorded his observations on the effects of exposure to high temperatures, of treatment with alkalis, and of filtration of the pitcher-liquid, upon its digestive activity.

The method of experiment with heat was to maintain the liquid for a given time at the required temperature, and then to institute a digestion experiment, adding fibrin and the necessary acid; in nearly every case there was a control digestion experiment with unheated liquids. The results were as follows:

\*The writer being very sceptical instituted some experiments and found the spores of the hay bacillus alive in pure glycerin in one case after 19 months, and in another case after 10 months. This spore bearing material was put into the glycerin in cotton plugged test tubes and tested for viability every few months, always with positive results.

Liquid maintained at 80° for 15 to 20 minutes did not complete digestion until the morning of the 4th day. The control required 3 hours. In another case digestion was complete in 24 hours. In liquid maintained at 78° to 83° C. for 30 minutes digestion did not take place within 4 days, and in that kept at 80° for the same time there was no indication of digestion within a week, while the control required only 5 hours. From this Vines concluded that the digestive power had been destroyed. Boiling for several seconds decreased but did not destroy the digestive power; for its complete destruction the liquid must probably be kept at 100° for 3 to 5 minutes.

Sodium carbonate was used exclusively in the experiments with alkali. To a quantity (5 to 10 cc.) of pitcher liquid an amount of the solid salt was added requisite to produce the desired alkalinity. After this the liquid was placed in the incubator, for a given time at a given temperature, then neutralized, then acidified with hydrochloric acid, and a digestion experiment was made.

Results proved to be dependent on the following three factors: (1) the degree of alkalinity (2) the duration of alkalinity, and (3) the temperature maintained during alkalinity.

Treatment with 0.5 per cent to 5 per cent sodium carbonate at 35° to 38° C. for periods varying from 30 minutes to 17 hours (the longer periods were used with the lesser degrees of alkalinity) always retarded digestion, though in every case it took place eventually. In the case of treatment with 5 per cent sodium carbonate for 3 hours digestion required 26 hours. This led Vines to experiment further with this degree of alkalinity, but at a higher temperature. He found that at 50° C. alkalinity for 45 minutes greatly retarded digestion, while exposure 1.5 hours destroyed the digestive power; i. e., there was no action within 6 days. Treatment with 1 per cent sodium carbonate for 1 and 1.5 hours gave results indicating that treatment with 1 per cent sodium carbonate for 1 hour at 50° C. is an approximate index to the stability of the enzym.

When pitcher liquid was passed through a Berkfeldt-filter it lost its acid reaction and its coloration, and proved far less active as a digestive agent. To test the value of this fact as evidence for the bacterial explanation Vines tested the effect of such filtration on liquids containing pepsin and ptyalin. These were affected in much the same way; the digestive power was very much reduced. Hence, if absence of the activity of pitcher liquid is due to removal of the bacteria, that of the gastric juice and saliva must be attributed to the same cause.

Vines states also that he has confirmed his results of 1877, when he demonstrated the presence of a zymogen in the glandular tissue of the pitcher. His experiments were conducted as follows:

He opened and washed out two unopened pitchers of *N. mastersiana* (the pitcher liquid was strongly acid), and cut up the glandular part into fine pieces. Half of it, A (8 grams), was rubbed up in a mortar with 20 cc. of distilled water; the other half, B, was rubbed up with 20 cc. of 0.25 per cent solution of hydrochloric acid. Both A and B were then placed for 45 minutes in the incubator at 50° C. The liquid was then poured off, the substance dried somewhat, with blotting paper, and then each was rubbed up with 20 cc. of glycerin. Eight days later digestion experiments were made with the glycerin extract with the result that the acid extract caused complete digestion within 8 hours while the neutral extract required 48 hours more. In another experiment, in which the material was treated with 0.5 per cent acetic acid at about 15° C. for 24 hours, the acid extract digested more rapidly than the neutral but the difference was not so marked as in the preceding case.

In a later experiment, the pitcher material was divided into three parts, two were treated as in the above experiment, and the third was rubbed up at once with glycerin. Digestion experiments were made with the glycerin extracts. In this case the tubes were kept in the incubator at 36° C. The fibrin in the acid extract had undergone solution in 3½ hours, that in the neutral extract required more than 3 days, while the third showed no sign of digestion at the end of 3 days.

From these results Vines draws the conclusion that a zymogen is present in the glands, from which an enzym is liberated on treatment with acid. He admits, however, that he has not always obtained these results. In some cases treatment with acid decreased instead of increasing the activity of the glycerin extract. This he thinks is due to the method of treating the pitcher material. The most effectual mode of decomposing the zymogen is to act upon the tissue with acid for a short time with a relatively high temperature (50° C.). He thinks also, that, while the acid of the pitcher liquid is useless for digestive purposes until the opening of the pitcher, it is probably of importance in that it acts upon the zymogen, liberating the enzym.

While investigating the nature of the ultimate products of digestion, Vines found indications of the presence of peptone, which he had before failed to demonstrate. By using Kühne's method for separating deutero-albumose and peptone he had no difficulty in demonstrating a true peptone. Ramsden, who had suggested this method, also found peptone present in the digestion products which he tested. The presence of leucin was confirmed.

In conclusion, Vines classifies the enzym concerned as a tryptic ferment, differing from the trypsin of the pancreatic juice in requiring an acid medium for its digestive action. It resembles that of the germinating seed, but is more rapid and energetic in its action.

In 1899, Clautriau published a very interesting paper on digestion in the pitchers of *Nepenthes*. He began his work on plants of *Nepenthes melamphora* in their natural habitat in the forest of Tjibodas on Mount Gedah, one of the volcanoes of Java. The following is an abstract:

*Nepenthes melamphora* is very abundant at an altitude of 1,500 to 2,200 meters, where the temperature is moderate, never exceeding 18 to 28 degrees at midday. Under these conditions digestion was much retarded.

He found that the use of cooked egg-albumen introduced bacteria and fungi. To avoid this source of error he diluted egg-albumen with 9 times its volume of distilled water, filtered it, and rendered it incoagulable by the addition of 0.1 mg. of crystallized iron sulphate per 100 cc., or 1 mg. in case of eggs not perfectly fresh. This liquid could be sterilized by boiling and introduced into the unopened pitcher under absolutely aseptic conditions, and also formed the basis for an exact comparison of the quantity of albumen digested in various experiments. As it kept indefinitely the same mixture could be used throughout. The iron sulphate used did not exert an injurious effect either on the pitcher liquid or the tissues of the pitcher itself. To render this fluid coagulable it is only necessary to add a little of an alkaline salt and to acidify very slightly.

As to the quantity of insect remains in the pitchers, Clautriau's observations led him to conclude that this depends on the abundance of insects in the locality in question. He found the pitcher liquid colorless, slightly viscid, insipid in taste, but with a slight odor, reminding one of certain honey, especially when the liquid contained insects. As the result of a great many observations he always found in unstimulated pitchers a litmus neutral liquid. He states that he drank the fluid from a great many unopened pitchers in his ascent of Gountour: "On eut dit une eau un peu mucilagineuse, mais sans le moindre saveur désagréable." Closed pitchers sometimes contained acid liquid, but this was due, he thinks, to a shock of some sort. He was able often to cause acidification in closed pitchers by shaking vigorously, the test for acidity being made the following day. Under natural conditions such stimulation might be caused by the wind, by birds, or by the movements of larvæ (mosquito, etc.) which sometimes reach adult form in the liquid. The introduction into the closed urn of any foreign body, even very slender pieces of drawn out glass tubing, provokes the secretion of acid.

The presence of uninjured larvæ in the pitchers does not, he thinks, exclude the presence of an enzym while it does testify to the absence of a toxic or anæsthetic substance. The fact that insects, falling into the liquid, were very slowly killed is another evidence against the presence of a toxin. He found that ants which had been immersed in the liquid half a day, recovered gradually when washed and dried somewhat. The captured animal is finally digested, only the chitinous parts remaining. The liquid remains absolutely limpid and without disagreeable odor, a proof that putrefaction has not taken place. This was also confirmed by use of the microscope. He found it impossible to add antiseptic substances to the fluid inside the pitchers without injuring them, even when these substances (formalin, chloroform, camphor, essence of mentha or citron) were used in extremely minute quantities. It is easy, however, by working aseptically on unopened pitchers to show that digestion takes place in the absence of bacteria and thus disprove the views of Dubois and Tschutkin.

The multiplication of bacteria in pitcher liquid (when cooked-egg albumen was used) appeared to be dependent on the amount of albumen added. Thus when only a small amount was present absorption by the pitcher kept pace with its digestion, affording unfavorable conditions of nutrition to bacteria. On the other hand, in liquid provided with a large amount of albumen, digestion was more rapid than absorption and bacteria developed rapidly. In no case was he able to obtain complete asepsis with cooked egg albumen. Moreover, much larger quantities of the fluid albumen were digested in a given time than of the coagulated albumen.

In his experiments with incoagulable albumen, Clautriau endeavored in vain to detect the presence of appreciable amounts of peptone. He found that in some cases the addition of albumen induced an acidity of the liquid corresponding to that of 2 cc. per liter of hydrochloric acid. The liquid became somewhat opalescent at first, later transparent, with an amber tint. Digestion was very rapid, but chemical analysis failed to demonstrate any products of digestion in the liquid though many experiments were made with varying amounts of albumen. When he had waited a sufficient time he also failed to find the original albumen. His conclusion is that the albumen is rapidly modified in the absence of bacteria, and that the products are absorbed as fast as produced.

He made, also, experiments in vitro to determine whether the rôle of the plant consists simply in the secretion of an acid and a zymase. The liquid from both open and closed pitchers was used. In addition to liquid albumen, one part received a few drops of chloroform, another was heated to 100°; the third received no treatment. They were left in the open, beside pitchers which served as controls. No digestion took place in the test-tubes though it was rapid in the controls. In only a single case did he obtain in vitro the disappearance of the albumen (almost complete) and the

production of albumoses. The liquid in this case came from a repeatedly nourished pitcher and was kept in the laboratory during the period of experiment (3 days).

To determine whether digestion was in any way dependent on absorption he separated pitchers from the plant at different intervals, after the addition of albumen, and in every case found that this separation from the plant inhibited digestion. A comparative study was made of the tissues of adult pitchers with and without nourishment by the addition of albumen. In adult pitchers which had received additions of albumen, the tissues in the vicinity of the spiral vessels coming from the glands and the tracheids which go to the vessels showed a manifest accumulation of proteids (De Wevre's cosine test).

Besides his researches in Java, Clautriau also worked on other species in hothouses in Europe, especially *N. mastersiana*.

For the separation of the products of digestion he used Neumeister's methods. His experiments were as follows:

He removed from a large pitcher the liquid containing the remains of insects, replacing it with a mixture of 12.5 cc. of distilled water and 2.5 cc. of incoagulable albumen. In all his hothouse experiments this albumen was used. The removed liquid (9 cc.) was filtered and divided into 3 parts. To *A*, 20 drops of albumen were added, to *B* the same amount of albumen and a drop of dilute chlorhydric acid (0.01 cc. HCl). *C* was kept in a hot water bath at 100° for 6 minutes before receiving the same treatment as *B*. A fragment of camphor was used in each case as an antiseptic. The tubes were kept in the thermostat at 37°. After 3 days, *A* and *B* contained no albumen, no syntonin, and only traces of albumoses; the peptonization was, therefore, complete. In *C* all the albumen had disappeared; there was much syntonin, a little albumose, and no peptones. This result was confirmed by many experiments. At a low temperature (20°) the same experiment gave different results. After 5 days there was a little albumose present and doubtful traces of peptones, while a considerable quantity of syntonin remained. Clautriau thinks, therefore, that temperature plays a large part in the proteolysis.

The experiments on absorption made in Java were repeated on hothouse plants with the same results. In one case the liquid of a pitcher was replaced with distilled water and albumen, on three different occasions. Each time the albumen was digested, and the products absorbed completely. In this way 32.5 cc. of the incoagulable albumen were digested without injury to the urn. Clautriau thinks that as the peptones are diffusible it is natural that they should be the first substances absorbed. In only two cases did he succeed in demonstrating peptones in the pitcher: Once in a pitcher of feeble vitality, once after adding methylene blue which seemed to retard absorption.

To prove that the plant really derives benefit from pitcher digestion, Clautriau undertook to show that the nitrogen of albuminoid substances was really absorbed by the plant and not present in the pitcher liquid in another form. The method used was to determine the quantity of nitrogen present in the pitcher, after a certain period of digestion with a known quantity of albumen. According to Kjeldahl's method, 10 cc. of incoagulable albumen gave a quantity of ammonia equivalent to 14 cc. of decinormal sulphuric acid. The same amount of albumen, after 7 days digestion in a pitcher, when subjected to the same treatment neutralized only 2.8 cc. of the decinormal sulphuric acid. In a second experiment it neutralized 2.7 cc. Thus after a week only 20 per cent of the nitrogen remained, part of which may have come from zymase and from the chitinous remains of insects.

Clautriau states also that the glands are the agents of absorption as well as of secretion. Microchemical examination showed that after digestion and absorption had taken place, the glands, and cells surrounding them, showed marked accumulation of albuminoid substances, while no such condition was found in the epidermal cells. Clautriau further states that he isolated a small quantity of true peptone. He failed to find either leucin, tyrosin, or amido-acids, and hence considers the enzyme a pepsin. Starch is not digested.

In 1901, Vines published another paper on the proteolytic enzyme of *Nepenthes*, first reviewing Clautriau's researches to which in some instances he takes exception, though on the whole he regards them as important.

In the first place Vines states that, contrary to Clautriau's inference, the addition of a little hydrochloric acid or of an organic acid hastens the process of digestion, although naturally acid pitcher liquid will digest proteid. Neutral pitcher liquid will not digest it at all. Moreover, with regard to the doubt expressed by Clautriau as to the presence of an enzyme in the liquid of unopened pitchers, he states that the liquid is very active when properly acidified.

The most important point of difference, however, is the nature of the enzyme, which Clautriau claims is a pepsin, on the ground that he has been unable to detect leucin or tyrosin: Vines on the other hand considers it a trypsin.

Desiring to obtain conclusive evidence for his claim, Vines undertook further researches. His former methods were unavailing in that he was unable to separate leucin or tyrosin in measurable quantities. His results, obtained by another method, he considers convincing. He says:

"Tiedemann and Gmelin observed in 1831 that on the addition of chlorine water to the liquid resulting from a pancreatic (tryptic) digestion, after acidification, the liquid acquires a color varying from pink to violet; when concentrated there is a violet precipitate. This coloration is due to the presence of a substance which, together with leucin, tyrosin, and other bodies, is a product of tryptic, as distinguished from peptic proteolysis. The substance in question is a chromogen termed *proteinochromogen* by Stadelmann, but better known by the name, *tryptophan*, given to it by Neumeister, and its presence affords a ready means of distinguishing tryptic from peptic digestions."

Vines's experiments using this method with the liquid from somewhat prolonged digestion of fibrin by pitcher liquid of *Nepenthes* in the presence of either hydrochloric or citric acid, gave the tryptophan reaction. He also obtained this reaction in liquids resulting from the digestion of fibrin by both pineapple juice and papain. These produce leucin and tyrosin in larger quantities than does pitcher liquid. There was no evidence of bacterial putrefaction, the products of which also have been found to contain tryptophan, *i.e.*, there was no odor of indol or scatol.

The action of nepenthin, as Vines calls this enzyme, was also tested on albumoses and peptones, with the result that the digested liquid gave the tryptophan reaction. Pineapple juice and papain thus tested reacted similarly. Controls of various sorts gave negative results.

Hence Vines concludes that the three enzymes, nepenthin, bromelin, and papain, have essentially the same proteolytic action, which is tryptic. Nepenthin, however, acts only in acid liquids, while bromelin and papain are most active in neutral liquids. Trypsin acts most readily in alkaline liquids. According to their mode of action, however, they may be grouped with trypsin. Vines thinks, also, that these investigations strengthen his suggestion that all known proteolytic enzymes of plants are tryptic.

#### BACTERIA IN HOP GLANDS.

In 1892, Mohl attributed the formation of lupulin in hops to the presence of a *Micrococcus*, called by him *M. humuli Launensis*. The organism is said to be present in enormous numbers in the glands of the living hop plant. Apparently no cultures were made, only microscopic examinations. No conclusive evidence was advanced. After reading his paper and especially after studying the glands of the hop-strobile one is prepared to appreciate Braungart's comment: "Sonderbarer Bemerkung." The glands are full of oil drops and of minute granules, some of which have an active Brownian movement when examined in water but do not stain like bacteria. The latter are probably what Mohl saw.

#### BACTERIA WITH ALGÆ.

Kozzowitsch in 1892 to 1894 obtained marked increase of nitrogen in mixed cultures of algæ and bacteria, but no increase in pure cultures of the algæ. He assumed, therefore, that the algæ and the nitrogen-fixing bacteria stood in a symbiotic relation to each other, the bacteria obtaining their carbon food from the algæ.

In 1900 Krüger und Schneidewind studied pure cultures of various lower algæ on a variety of culture media with and without combined nitrogen, and reached the conclusion that these algæ were unable to obtain their nitrogen from the air. Their paper does not deal directly with the question of the relation of these algæ to the nitrogen-fixing bacteria of the soil.

In 1903, Reinke published a paper on the symbiosis of *Volvox* and *Azotobacter*, in which he presented additional evidence in favor of the theory that *Azotobacter* furnishes nitrogenous compounds to fresh water algæ, as well as to the salt-water forms, with which it is associated, and to the outer membranes of which it adheres closely ("so fest eingenistet sind, dass ein Zellenverband von gewebeähnlicher Innigkeit entsteht.")

At his request Keutner made a large number of cultures of fresh water algæ from the plankton of Lankener Sea near Preetz, as well as from the ponds of the botanical garden. The cultures, left to themselves during the summer vacation, showed in October a great development of *Azotobacter*, and had furnished in the 200 cc. of nitrogen-free nutrient solution an appreciable amount of combined nitrogen, on an average, about 1 mg.

As an example of these experiments the culture of *Volvox* is given in detail.

By careful washing, the spheres of *Volvox* were freed from pond water and placed by means of a sterile platinum loop in a sterile nutrient solution consisting of 200 cc. of water containing 4.0 mannit, 0.1 of potassium phosphate, 0.05 of magnesium phosphate, and 0.3 of calcium carbonate. At the end of 10 weeks this solution contained 11.6 mg. of combined nitrogen. The only possible source of this nitrogen was the assimilation of the atmospheric nitrogen, absorbed in the water. *Azotobacter* had developed abundantly.

The infection with *Azotobacter* was possible only through the introduction of *Volvox* to the surface of which some of these organisms were clinging.

Reinke's conclusion is that *Azotobacter*, while drawing its carbon in organic form from the *Volvox*, furnished the latter with nitrogen compounds. He is convinced that this theory concerning the source of nitrogenous compounds for both fresh and salt water algae is worthy of preference over every other hypothesis. As a further support for his theory he mentions the fact, discovered by Gerlach and Vogel, that the dry substance of *Azotobacter* contains 10 to 12 per cent of nitrogen.

In 1904, Hugo Fischer published a short article on an assumed symbiosis between *Azotobacter* and *Oscillaria* living on the ground.

He obtained samples of dark green *Oscillaria* from different localities, and covered them with a 1 per cent solution of mannit, according to Beyerinck's method. There was such a rapid development of *Azotobacter* in pure culture that he was forced to assume an especially favorable growth of it among the filaments, although he could not demonstrate it microscopically. From this common occurrence together, he concludes that a symbiotic relation exists by which the bacteria furnish nitrogen compounds to the algae taking in return from their supply of carbohydrates. The earlier assumption, therefore, that the lower algae are able to assimilate free nitrogen should, he thinks, be retracted.

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## BACTERIAL SYMBIOSIS IN CRYPTOGAMS.

## BACTERIA WITH YEASTS.

## KEFIR.

Kefir is a granular gelatinous substance, horny when dry, occurring naturally in the Caucasus. It has been known for centuries and used extensively to make a fermented drink from milk—alcohol, carbon dioxide, and lactic acid being produced. The substance is said to occur naturally on peculiar bushes just below the snow line in the mountains (Mix) but this is probably folk-lore. Gradually a knowledge of the substance diffused into Europe and it may now be had in many places. Beyerinck states that it is identical with the ginger-beer plant of England, the kefir having been brought back from the Crimea by the English soldiers. Beyerinck's figure of kefir differs so much, however, from the figures and description of the ginger-beer plant published by H. Marshall Ward that they would seem to be two different substances. Many papers have been published on kefir. The chemistry of the action appears to be better known than the specific organisms which enter into the composition of kefir.

Kern was the first botanist who called general attention to the morphology of the substance. He found it to be composed of yeast and bacteria in what he believed to be a symbiotic relationship. He considered the yeast to be the common beer yeast, *Saccharomyces cerevisiae*. He described the Schizomycete as *Dispora caucasica*. This schizomycete, he stated, commonly produces a spore in each end of the rod, the diameter of this spore being not greater than the rod itself.

The substance of Kern's paper is given in the following abstract:

Kern observed kefir in the Caucasus in the summer of 1881. The mountain people make large use of milk as food, but they do not use the milk to any great extent in a fresh condition, first fermenting it in leathern sacks by means of grains of the kefir. The kefir fermentation goes on more rapidly if a large number of grains are put into the milk. Ordinarily the kefir is ready to use in a few hours, the leather sack first being shaken thoroughly before it is poured out. After the kefir is poured out of the sack the latter is filled with fresh milk.

"When the kefir preparation has succeeded it is a thick fluid mass without any large coagulated lumps, agreeably sourish in taste. By a longer fermentation it is converted into a mossy, foamy, strongly acid drink, which is similar to the Kumys of the Steppe region."

The dwellers in the mountains use the kefir not only as food, but also as a medicine in various diseases with results, believed to be superior to those obtained with Kumys. Kern states that he was unable to find any scientific data on the subject. He states that the physiological and therapeutical action of the kefir is a thing to be studied by a physician. He devoted himself to an examination of the microscopic structure of the kefir grains and the morphological nature of the ferment.

"The little clumps form white, compact, elastic masses, covered over with slime. They have a spherical or elliptical form and a size of 1 mm. to 5 cm. Quite small lumps have a smooth, spherical exterior, the larger on the contrary, are provided with various protuberances and furrows (fig. 38) and in appearance are not unlike a cauliflower head."

In every such lump, no matter what its form or size, the microscope shows two different structures that is, yeast-cells and bacteria. The yeast-cells are inclosed in groups here and there in the mass of the bacteria.

For his mass-cultures Kern used nutrient fluids, at first Pasteur's fluid, composed of 1,000 grams of water, 100 grams of candy sugar, 1 gram right ammonium tartrate, and the ashes of 10 grams of yeast. Subsequently he confined himself exclusively to Cohn's normal bacterial nutrient fluid (Untersuchungen, Bd. I, Heft 2, p. 196), and like Eidam recommends substituting for the insoluble tribasic phosphate of lime, equal quantities of calcium chloride. This fluid is only adapted to the nourishment of the bacteria. For the needs of the yeast he added milk-sugar, in about the quantity found in milk. The nutrient fluid which he used for the most of his cultures had, therefore, the following composition:

Distilled water.....	1000
Milk-sugar.....	44.5
Ammonium tartrate.....	9
Calcium chloride.....	0.5
Magnesium sulphate.....	5
Phosphate of potash.....	5

This fluid is transparent and slightly acid in reaction.

To learn the microscopic structure he took single fresh lumps out of milk, washed them carefully in water, then teased out portions with a needle and examined under a microscope fresh in water. He also used various stains, such as carmine, picrocarmine, hematoxylin, eosin, purpurin, fuchsin, aniline blue. Of these strains he found eosin best for the yeast-cells and fuchsin for the bacteria. For more exact study of single forms he made use of cultures in hanging drops in Faminztzin's moist chamber.

Kern states that the yeasts occur as single cells, pairs, and rows of cells, and are extremely variable in form and size. He found them for the most part elliptical or spherical, but in nutrient fluids he also often observed cylindrical cells or polygonal cells.

The larger diameter of the elliptical cells varied between  $3.2$  and  $9.6\mu$ , the smaller between  $3.2$  and  $6.4\mu$ . The spherical cells were  $3.2$  to  $6.4\mu$  in diameter, each cell had a plainly double contour membrane. The protoplasm enclosed a vacuole. On the poles of the vacuole small dots of fat were often to be seen. The well-nourished, normal cells reproduce by budding. Although he cultivated his yeast-cells for weeks on potato and carrot, and pieces of black bread, he could never find any outgrowth of the same into threads. He, therefore, concluded that he had to do with a genuine yeast and not with any form of *Mucor*. He was not able to induce this yeast to produce spores,

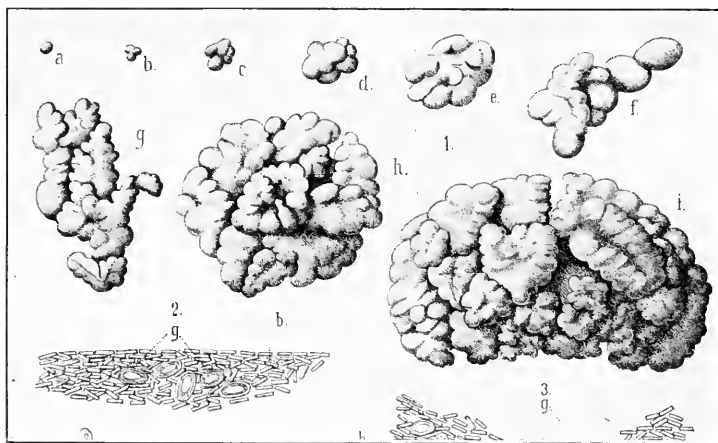


Fig. 38.\*

although he carefully followed the directions of Dr. Max Rees and Emil Schumacher. His cultures remained *in statu quo* for weeks and then finally perished without formation of spores. He attributed his failure to obtain spores to the fact that he had to do with the common cultivated beer yeast, *Saccharomyces cerevisiae*. He states that in fermented milk the kefir grains sink to the bottom. On the contrary in fresh milk they rise immediately to the top and remain there during the whole time of the fermentation. [Buoyed up undoubtedly by the liberated gas.]

Concerning the bacteria necessary to the ferment of the kefir grains Kern makes among others the following observations. The vegetative bacteria have the form of short cylindric rods  $3.2$  to  $8\mu$  long by  $0.8\mu$  broad. The inner structure of the cells appeared to be uniform, no inclusions having been observed. The rods also may be separate or remain attached for a long time, in which case they may form long threads. In the clumps of kefir one has unquestionably to do with bacteria in the state of zoogloae. Chloriodide of zinc does not react on the slime. In addition to the resting zoogloae stage, there is also a stage of motile cells, which cannot be distinguished from the other stage in form or size. These latter are provided with one polar flagellum. The flagella were demonstrated by Koch's early method, viz., by staining them for some time in extract of campeachy wood. Exposed to the action of alcohol, Müller's fluid, various acids, or to drying, high temperature and lack of food, the vegetative bacterial cells of the clumps are said to grow out into long *Leptothrix*

\*FIG. 38. (1) Kefir grains; (2) margin of a grain magnified to show relation of bacteria to yeasts. After Kern.



threads, individual joints of which are readily demonstrated by the use of fuchsin. He states that he observed threads from 10 to  $40\mu$  long. Most of these were not straight but wavy and bent. They form commonly an interwoven, felt-like layer. The growth of *Leptothrix* threads due to unfavorable conditions usually precedes spore formation. In such threads there is a row of spores, while in the single vegetative cells which do not grow out into threads, there are always only two spores in a cell, one at each end (fig. 39). The spore formation begins with the appearance at each end of the cell of a small, bright dot, which gradually increases in size, becomes bounded by a sharp contour and is finally converted into a true spore. These spores are always round and their diameter never exceeds the thickness of the cell. The figure borrowed by von Freudenreich represents not spores but germinating spores. The shortest cell observed with two polar spores measured  $3\mu$ . Most of them were  $6\mu$  long. The longest seen was  $20\mu$ . He was never able to find any cross-wall separating the two spores, not even when he used Hartnack Imm. X. He, therefore, concludes that the two spores are certainly inclosed in one cell. He could not make out in the vegetative cells whether the spore formation was brought about by free cell-formation or by cell-division. On the contrary, in the *Leptothrix* threads he found a plain cell-division. The round free-lying spores reach a diameter of  $1\mu$ . The germinating spores swell up to a diameter of  $1.6\mu$ . He was able to observe the germination and has figured it, but it is not perfectly clear from his statements whether these germinating spores were those from the *Leptothrix* threads, or those from the motile organism or from other non-motile short rods or whether they really had anything to do with the organism concerned in the kefir symbiosis. After considerable discussion of the views of earlier writers on the systematic position of the bacteria, he describes his organism, *Dispora caucasica* as follows:

"Vegetative cells in the form of short cylindric rods,  $3.2\mu$  to  $8\mu \times 0.8\mu$ . In zoogloae condition the cells form white compact elastic clumps of considerable size (up to 5 cm.). The motile vegetative cells have at one end a thin thread-like, wavy flagellum. The spores are round. Lying in the cells they do not exceed the breadth of the latter. When they are free they are  $1\mu$  in diameter. The round spores are always arranged two in a cell, one at each end."

The kefir clumps do not appear to lose power of growth by drying. They shrink considerably, become dirty brown and stone hard, but are able again to resume their activity when thrown into milk, and are preserved by the mountaineers in a dry condition for a long time. The author himself preserved them in an air dry place for two months, and after a few days, when thrown into milk these could not be distinguished from fresh clumps nor was there any perceptible difference in their power of fermentation. Under the microscope the dry clumps showed a considerable number of changes. Many of the yeast-cells were dead and those which remained alive were principally spherical. These dried ones contained no spores. The *Dispora* when in spore condition is said not to be destroyed by boiling for an hour.

The foregoing is the substance of Kern's paper in the Bulletin de la Société Impériale des Naturalistes, Moscow, 1881. He sums up his conclusions as follows:

- (1) The little clumps, the ferment of the Kephir, afford an interesting example of a symbiotic life—commensalism (?)—of yeast-cells and bacteria.
- (2) The yeast-cells are to be considered as the ordinary beer-yeast, *Saccharomyces cerevisiae* Meyen.
- (3) The bacteria, in the vegetative condition scarcely to be distinguished from *Bacillus subtilis* Cohn, may, on the ground of very peculiar spore formation, be set off into a new genus, near the genus *Bacillus*—*Dispora caucasica*, nov. gen., nov. sp.
- (4) A distinct cell-membrane can be distinguished on the vegetative cells of the *Dispora*.
- (5) The motile cells of the *Dispora* have a thin, thread-like, wavy flagellum at one end.
- (6) Moreover, the little clumps, but especially the vegetative cells and the spores of the *Dispora*, are very resistant to unfavorable influences.

This paper is followed by two tables of figures. From them I have borrowed the two figures 38 and 39.

\*FIG. 39.—Spore formation in *Dispora caucasica*, and also mature and germinating spores (25). After Kern. In his fig. 23 at p, p, are masses of protoplasm which he states he observed to break up into two spores. In his figure 24 are a group of vegetative cells provided with a spore at each end and destitute of any cross-wall between them. In his fig. 23, one, two and three are said to be stages following each other in spore development.

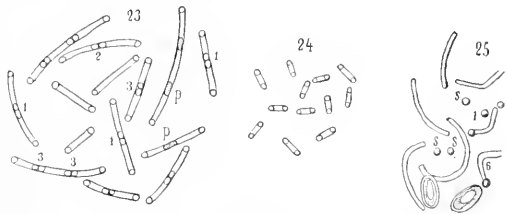


Fig. 39.\*

Lewton-Brain and Deerr have published a figure strikingly similar to Kern's figure here listed as 39 (The Bacterial Flora of Hawaiian Sugars, Bull. 9, Exp. Sta., H. S. Pl. Asso. Honolulu, 1909, p. 21, fig. 17). This was drawn from their *Bacillus D*, a sugar destroying species. It is called by them a curious bipolar effect produced by carbol fuchsin staining.

[This] consists of a faintly stained central part, with a very brightly stained circular body at each end. \* \* \* It seems possible that the central body represents the spore, the two brightly staining bodies the degenerated protoplasm of the remainder of the cell, while the walls of the cells have swollen up and become confluent with those of other cells lying close at hand. This peculiar effect was not met with in the other bacteria stained in the same way, and was always obtained with *D*, so that it would appear to have some diagnostic value.

Beyerinck devoted a paper to this subject in 1889.

Kefir is composed of a yeast and a schizomycete in symbiotic relationship, the result of their combined action on milk being alcohol, carbon dioxide and lactic acid. He distinguished the yeast as a new form, *Saccharomyces kefir* and described the schizomycete as *Bacillus caucasicus*. These together produce small plates which grow by the formation of local excrescences that increase in size and fuse at their base. In sour milk the kefir grains remain alive for a long time. He figures the yeast as occurring in a uniform thin layer on the outside of the grains. He also states in the text that the yeast occurs almost exclusively on the surface of the grains, the bulk of the mass being bacteria. In some instances, however, he states that he saw chains or layers of the yeast in the interior of the mass. In the bacterial mass Beyerinck distinguished a cortical layer, and a so-called pith, with a central cavity partly or fully occupied by zoogloæ masses.

The yeast is oval. The measurements given by Beyerinck are 3 to 6 $\mu$ . It is easy to cultivate. It is able to convert milk-sugar into alcohol and carbon dioxide. After a long time it liquefies neutral or feebly alkaline lactose gelatin. This yeast tolerates a large amount of lactic acid (up to one-half normal), acetic acid on the contrary is very injurious to it.

The yeast is said to invert milk-sugar by means of an enzyme, lactase. That the inversion precedes the formation of alcohol was shown by the use of poured plates containing fish bouillon, 3 per cent sea-salt, and 7 per cent gelatin, to which was added some milk-sugar and then sown with his *Photobacterium phosphorescens*. After 2 days the gelatin became luminous. Later, as the food was exhausted the luminosity diminished, this organism being unable to use the milk-sugar present in the gelatin. If then the kefir yeast was placed on portions of the plate these portions again became luminous, indicating the liberation by the yeast of simpler, assimilable sugars from the milk-sugar.

The bacillus produces lactic acid. It is mixed with other bacteria which are to be regarded as impurities. Some of these impurities are readily distinguished, *i.e.*, the bacilli producing carbon dioxide and hydrogen, or lactic acid milk ferments in the form of diplococci, *Oidium lactis*, and foreign yeasts. Other rod-shaped bacteria are less easily distinguishable, such as those producing acetic acid or lactic acid. The advantage of the symbiosis to the yeast is freedom from acetic acid and from putrefactive bacteria, which are assured to it by the presence of the lactic acid ferment. The advantages to the bacteria from the presence of the yeast are less clearly evident, but are believed by Beyerinck certainly to exist. He offers some hypotheses, but no actual facts.

A good culture medium for *Bacillus caucasicus* is gelatin with serum of milk either neutral or feebly acidified with lactic acid. When such a medium is sown with water in which a fragment of kefir has been crushed, there appear after two or three weeks, between the yeast colonies, very minute and extremely slow-growing colonies of this lactic ferment. The most favorable temperature for the growth of these minute colonies is said to be 45° C., and then, of course, agar plates must be used. *Bacillus caucasicus* does not liquefy gelatin. It forms neither lactase nor invertase, but transforms milk-sugar, cane-sugar, maltose and glucose directly into lactic acid. The optimum temperature for this change is 40° to 45° C. There is no special action on starch or casein. The fermentation and formation of lactic acid take place equally well in the presence or absence of free oxygen.

"Under all conditions the ferment keeps the form of rods or filaments, which often remain united into chains and may become very long. I have never observed the least indication of spore formation, nor of motility."

Beyerinck was not able to obtain the kefir synthesis by using a mixture of the yeast and lactic ferment on lactose gelatin.

In 1891, Mix published a contribution upon an American kefir. The work was done at Harvard University on material obtained from two different sources, *i. e.*, New Jersey and Ontario. Both specimens were lobed and fissured and of a dirty brown color resembling

ling dirty gum arabic. That from New Jersey had been dry for more than two years but revived when placed in a nutrient fluid and most of the studies were made with this New Jersey form.

The yeast in this kefir Mix states to agree with *Saccharomyces kefir*, and to differ from *Saccharomyces cerevisiae*. It agrees with Beyerinck's form in measurements, in being associated with a rod-shaped schizomycete in a granular mass, in being able to ferment lactose, but not saccharose, and in not producing spores. "Although I cultivated it in saccharose solutions of all strengths, it never caused a trace of fermentation." It ferments milk. The cells of the yeast were of various sizes and shapes, from spherical to elliptical, the spherical ones measuring  $3.2\mu$  to  $6.4\mu$  in diameter, the elliptical ones varying from  $3.2\mu$  to  $9.6\mu$  in the major axis by  $3.2\mu$  to  $6.4\mu$  in the minor axis. The bacteria are described as short symmetrical rods, varying from  $8.5\mu$  to  $4.5\mu$  by  $0.8\mu$ , precisely agreeing with Kern's measurements.

"The cells increase by splitting perpendicularly to the long axis, the resulting cells being sometimes joined together, thus producing leptothrix-like threads of all lengths, even to  $120\mu$ , and sometimes completely separated. Many of the isolated cells possess the power of motion, but after repeated efforts I was unable to demonstrate the presence of cilia."

He states it is not easy to induce these bacteria to produce spores, but that he was able to observe spore formation by placing a clump of the yeast [Kefir grain] in a watch crystal with a little water and covering the whole with another crystal. In 24 hours the threads began to form and within 36 to 48 hours the spores appeared.

"It will be remembered that Kern gives two distinct methods of spore formation—one occurring in isolated cells, the other in the leptothrix-like threads. \* \* \*

"My investigations on the North American form have led to results diametrically opposed to those of Kern. First, I found but one method of spore formation; secondly, I found this method occurring only in the leptothrix-like threads, although I sometimes found isolated threads bent or curled in such a manner that spore formation was well simulated. Spore formation in the leptothrix threads takes place as follows: At each end of each cell of the thread a small bright dot appears. It becomes brighter, larger and much more highly refractive than the rest of the cell until finally it assumes a well-defined spore wall and develops into the mature spore. Each cell has produced two spores, one at each end, and each originating independently of the other. In no case did I see two spores formed, as Kern states, by the division of a single agglomerated mass of protoplasm into two portions."

With this kefir-like substance Mix obtained alcoholic fermentation of milk with the formation of carbon dioxide and lactic acid, and the production of a fermented milk closely resembling the descriptions of kefir.

"The milk does not sour in the ordinary sense, for it does not coagulate in large masses; still it is acid, contains some carbonic acid gas and alcohol, and is by no means unpleasant to the taste."

Mix further states that the North American form of kefir causes (1) alcoholic fermentation of milk sugar; (2) the alcoholic fermentation of dextrose, and (3) that it does not cause the fermentation of cane-sugar. He thinks that the alcoholic fermentation of the milk takes place in the following manner:

"The *Bacillus acidi lactis* begins the process by forming some lactic acid, which in turn, assisted by the bacillus itself, inverts the milk-sugar to galactose and dextrose. The galactose is further acted upon by the *Bacillus acidi lactis*, and converted into lactic acid; the dextrose is acted upon by the yeast, and converted into alcohol and carbonic acid gas. In the kefir drink, therefore, we should find plenty of lactic acid, a little milk-sugar, not inverted, the amount depending upon the duration of fermentation, some alcohol, and carbonic acid gas—precisely what is found."

In 1896, Ed. von Freudenreich published a paper on kefir, of which the following condensation includes the most important statements:

From his own experiments which are in harmony with those of all previous observers he concludes that Beyerinck did not have the kefir yeast because this yeast is unable by itself to ferment milk-sugar. From Beyerinck's drawings and from the trouble he had in isolating the *Bacillus*, it seems probable to von Freudenreich that Beyerinck had under observation the same bacterial organism that von Freudenreich has studied and which he is inclined to consider Kern's *Dispora caucasica*, with, however, a considerable number of reservations as to its morphology. He thinks that the motile one-flagellate bacteria described by Kern probably had nothing to do with the *Dispora*. Other observers he states have come to the same conclusion, for example, Adametz. The *Bacillus subtilis* which frequently occurs in the kefir grains has nothing to do with the kefir fermentation according to

Essaulof, with which conclusion von Freudenreich agrees. Essaulof believed that only *Bacillus acidilactici* and the yeast were necessary to the formation of the symbiosis. Von Freudenreich was unable to obtain conclusive evidence on this point. He found other lactic acid bacteria and suggests that possibly the same micro-organisms do not always occur in kefir.

"The yeast and Kern's *Bacillus* are always present but the lactic acid bacteria may possibly be different if only they can bring about the splitting up and fermentation of the milk. If one sums up the results obtained hitherto we have in kefir an example of the symbiosis of several micro-organisms, among which is a yeast that according to most authors is not able to ferment milk-sugar, as well as probably a lactic acid ferment and a bacillus hitherto only cultivated by Beyerinck, which appears to be identical with the bacillus present in the kefir grains and described, but not cultivated by Kern. On the other hand, up to this time the rôle of these particular micro-organisms in kefir fermentation has not been clearly made out."

His own experiments began in 1892 and were continued with interruptions up to the time of the publication of his paper. In his preparations he found especially yeast-cells and long, mostly bent bacilli, very much resembling Kern's pictures, but also shorter rods—younger stages of the bacillus—and furthermore, coccus forms, the latter, however, much more rarely. Often he found that the bacillus stained only at the two poles, a phenomenon which he thinks led Kern into error as to the presence of spores in his *Dispora*. He thinks that the sporogenous organisms occurring in Kern's cultures were only potato bacilli and similar bacilli. "I have never observed spores in the bacillus of the kefir grains, *Dispora caucasica*, therefore, I would write *Bacillus causicus*."

When the kefir was clean, von Freudenreich found four different micro-organisms in it, namely, yeast cells, large coccus forms arranged in chains, smaller cocci and bacilli. The larger streptococcus and the yeast grew readily on gelatin plates, sometimes also the smaller streptococcus, but not the bacillus. Only once did he obtain colonies of the latter on an anaerobic gelatin plate. On the surface of milk serum agar plates at 35° C. one readily obtains the smaller streptococcus along with the larger one and also very small colonies of a bacillus believed to be identical with *Bacillus causicus*. Nevertheless he says that such cultures do not always succeed. Sometimes its colonies were entirely absent without any reason therefor being apparent. When he made streaks on slant milk serum agar, using kefir itself as a substance for inoculation and keeping the tubes at 22° C. he states that he obtained masses of growth containing the four organisms mentioned, and he figures the microscopic preparations of such mixed cultures, but these figures are not very conclusive as to any symbiosis. He says also that *Bacillus causicus* may be isolated by stab-cultures in deep layers of agar, these cultures being kept at 35° C. In the stab then appear mostly only the bacillus and the small streptococcus.

He describes the yeast as follows. *Saccharomyces kefir* is obtained readily in plate-cultures where it produces very small, coarse-grained pale colonies. On milk serum gelatin plates the colonies are said to be round and yellowish and better developed than on ordinary nutrient gelatin. The granulations on the edge of the colony are coarse. On the less thickly sown plates, the superficial colonies are well formed and whitish, finally yellowish. The buried ones are a yellowish color. The center of the colony is dark brown. Stab-cultures were distinctly visible in 24 hours. Development on the plates also was rather prompt, the colonies being visible for the most part after 2 to 3 days. Beef-broth kept at 26° C. clouded in 24 hours, also milk-sugar bouillon. The growth, however, is not so vigorous as in beer-must. In the latter medium there was an abundant growth. Gas-production occurs but is less abundant than in case of beer yeasts. Maltose is fermented by the yeast. The yeast ferments grape-sugar with production of alcohol. It does not cause any fermentation of milk, but develops well in it with the formation of a peculiar taste, which is different from that due to beer yeast. To the eye the milk remains unchanged. On potato the yeast grew with the formation of a yellowish patch. The optimum temperature is about 22° C. The yeast will grow at 28° C., but not at 35° C. This yeast consists of oval cells of variable size, on an average 3 to 5  $\mu$   $\times$  2 to 3  $\mu$ . Single cells are roundish, especially in potato cultures. The cells stain readily with all the ordinary aniline dyes, also by Gram. There is ordinarily a vacuole. In the protoplasm there are one or more shining granules. The yeast is unlike *Saccharomyces pastorianus* 1, II, and III, and also unlike *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus*, with which he compared it. There is never any pellicle formed by the kefir yeast, something which always goes with the other yeasts mentioned. He could not discover any ascospore formation. A temperature of 50° C. for 5 minutes sometimes sterilizes the culture, and a temperature of 55° C. always sterilizes it. He found the yeast also very sensitive to dry air. It endured 2 and 3 days' exposure, but not 4 or more days when taken from fluid cultures and exposed on filter paper. I omit descriptions of the two forms of streptococci because most observers are agreed that they only occur accidentally in the kefir. The photomicrograph of his larger streptococcus shows an organism with a long diameter nearly double the short diameter and makes one think that very likely the organism figured is not a streptococcus at all.

*Bacillus caucasicus* is described as follows: On ordinary gelatin plates it does not grow at all. Only once, as already stated, did von Freudenreich obtain it on a gelatin plate exposed to anaerobic conditions according to Miquel's method. Other times, using the same method, he did not obtain it. Also on milk-sugar gelatin plates he never observed it. Having once obtained it, it grows in stab-cultures even in ordinary gelatin but then first after a long time. On milk-sugar gelatin plates he often had no growths; at other times microscopic colonies. On the surface of milk agar plates, on the contrary, he often obtained colonies. Upon this it produces small, flat, grayish colonies which appear circular to the naked eye. With a weak magnification they are seen to have irregular contours and are not uniformly circular; they also appear whitish and granular. This granulation is produced he states, by the irregular arrangement of the bacilli, plain to be seen on the edge, out of which the bacillary forms project. In ordinary nutrient bouillon he could not obtain any growth, not even at 35° C. In milk-sugar bouillon there was a slow growth at 22° C.—nothing to be seen for the first 3 days, but at 35° C. the growth is faster. The reaction was acid. It produces no coagulation in milk although the reaction becomes somewhat acid. The taste of such milk was slightly acid and astringent similar to that produced by the smaller streptococcus when grown in milk. There was a moderate gas formation. There was no growth on potato. In milk-sugar bouillon it appears ordinarily as a straight bacillus with rounded end, often with a shining point at each end. This appearance corresponds well, he says, to the phenomenon interpreted as spores by Kern. Their slight resistance to heat, however, shows that they are not spores; also when exposed to staining media the bacillus stains in toto which would not be the case if these bodies were spores. The organism stains easily with the common aniline dyes, and also by Gram's method. The breadth of *Bacillus caucasicus* is about 1  $\mu$ , the length 5 to 6  $\mu$ , but long forms are also found which are then crooked. It is very feebly motile. A good photomicrograph of this organism is shown in his fig. 5, table 1 (fig. 40). Its resistance to external influences is slight. It endured drying 1 day. It was regularly killed by a drying of two or more days. Nevertheless, it lives a long time in the kefir grains, which he thinks is explainable by the fact that it is protected from the action of the air. It was killed, as already stated, by 5 minutes exposure to 55° C., while 2.5 per cent carbolic acid killed it in 30 seconds. Corrosive sublimate 1 : 1000 killed it in one experiment in 1, 2, and 60 minutes, but not after 5 and 15 minutes. This contradiction is attributed to dissimilar resistance of individual bacilli. The acid was estimated in terms of lactic acid, but I find no statements concerning its determination.

When inoculated separately into milk he could not obtain with cultures of these organisms anything corresponding to kefir. Moreover, with two organisms alone he could not obtain kefir. The yeast and the large streptococcus caused the milk to coagulate with a small amount of gas formation but no further change. The small streptococcus combined with the yeast and inoculated into milk produced gas formation and a sour taste, but no kefir fermentation. The amount of gas formed was variable. This he attributes to decrease in the virulence of the streptococcus. Finally, with the yeast and the *Bacillus caucasicus* he could not obtain kefir. Also when he inoculated all four of the micro-organisms together the experiment miscarried regularly at the beginning. The lactic acid ferment took place, the milk coagulated, but nothing further happened.

Von Freudenreich states that throughout his studies he had many failures but that finally he frequently obtained good kefir by inoculating milk with mixed growths of the organisms obtained by rubbing kefir grains on slant agar. Usually the first flask of milk inoculated did not yield kefir, but when transfers were made from this to a second flask of milk good kefir was often obtained. He seems to have been more successful in using this source of inoculation than pure cultures of the separate organisms mixed, although on the second or third transfer from milk to milk he states that he also obtained kefir from these. He states that sometimes he obtained a drink which could scarcely be distinguished from kefir by use of the yeast and the two streptococci.

He was never able to produce the kefir grains synthetically, and he considers that the rôle of the *Bacillus caucasicus* is still involved in a good deal of uncertainty. Its presence seems absolutely necessary to the symbiosis, but just what its function is in the fermentation he does not know.

Podwyssotsky (French edition of 1902) says nothing is known respecting the origin of kefir. There are various hypotheses current among the natives of the Caucasus respecting it: (1) The kefir grains are the direct gift of God through his Prophet Mohammed,

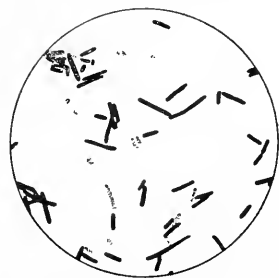


Fig. 40.\*

\*FIG. 40.—*Bacillus caucasicus*. From a photomicrograph by von Freudenreich.  $\times 1000$ .

and hence called "Millet of the Prophet;" (2) the grains were found very long ago in a bush on the high mountains near the eternal snow; (3) the first grains appeared in a dirty milk receptacle (outre). "Cette dernière version populaire se rapproche beaucoup, à notre avis, de la vérité."

According to Podwysotsky, the kefir grain is composed of three organisms: The kefir bacterium proper, the yeast, and a third schizomycete which produces the lactic acid. He states that Stanghé was the first to call attention to the presence of a third organism in kefir. The yeast cells are on the outer face of the grains. The deeper layers consist of a fibrous stroma of bacteria. This author states that healthy kefir grains never contain streptococci nor staphylococci. He is inclined to consider the the kefir bacteria as related to *Bacillus subtilis* (descended from it). He states that the yeast resembles *Saccharomyces cerevisiae* in its action. Moreover, if the alcoholic action of the kefir in milk is not proceeding properly it may be hastened by the addition of ordinary beer yeast. Podwysotsky also refers to the fact that there appear to be two types of kefir grains; a coarse large form which comes to the top of the milk during fermentation, and a smaller grained form which lies at the bottom. These have the same action on the milk. The grains which occur at the bottom of the milk break apart more easily when pressed between the fingers and are not as elastic as those which float.

The kefir grains, especially as brought into the market dry, are often attacked by other organisms *e. g.*, *Oidium lactis*, *Penicillium glaucum*, coccus forms, and various rod-shaped bacteria. A cursory inspection of these grains is often sufficient to show that they are diseased by these extraneous organisms, the surface of the dry grains being covered with white spots. If the grains are dried slowly in a moist and shady place, they often become very moldy and exhale a characteristic and very disagreeable odor.

He recognizes especially two diseases of the kefir fermentation:

(1) Mucification of the grains due apparently to the multiplication of foreign bacteria, the yeast cells being destroyed, and spherical and long filamentous bacteria becoming abundant. This is believed to be a contagious disease since, if a single affected grain occurs in a mass of grains, there will be many others after some days.

(2) A butyric acid fermentation which may be readily detected by the peculiar penetrating odor, resembling that of rancid butter. Moreover, microscopic examination of a drop of the fermented milk shows the presence of a great number of bacteria with swollen ends while the yeast cells have here also disappeared.

On account of the prevalence of extraneous molds and bacteria, kefir grains designed for sale dry should be washed thoroughly in several waters, *i. e.*, until the water comes away clear, and then dried rapidly in the sun on linen or filter paper.

Podwysotsky states that the kefir ferment may be obtained in various places in Europe in the form of tablets and powders. These are not so efficient as the kefir grains, but by several transfers through milk the kefir ferment may often be obtained from them in an active condition. The first product is usually too acid and does not contain enough carbon dioxide and alcohol. Much of the kefir on the markets in Russia is contaminated by butyric acid organisms and is of very inferior quality. Many of the kefir grains offered for sale are also of this character.

The best temperature to obtain a suitable kefir fermentation of milk is stated to be 15° to 17°C. At temperatures of 25° to 30° C. the lactic acid fermentation is too intense and only insignificant quantities of alcohol and carbon dioxide are produced. Frequent agitation of the receptacle containing the fermenting milk is considered to be very desirable; more so, even, than in the case of Kumys. The author states further that the inoculated milk should be left open to the oxygen of the air for the first 6 to 8 hours, then closed tightly and the fermentation allowed to continue for 1 or 2 days. The finished kefir should contain about 0.7 to 0.9 per cent lactic acid, a small amount of pectone, 1.5 per cent or less of alcohol and considerable quantities of carbon dioxide. Kefir more than 5 days old should never be consumed. A drop of good kefir 2 days old under the microscope should contain some yeast cells, considerable numbers of kefir bacteria, numerous minute lactic acid bacteria, a fine deposit of precipitated casein, and fat drops of various sizes. Kefir grains moistened and rubbed upon a slide should show under the microscope yeast cells, large bacteria (the specific kefir organism), and smaller lactic acid bacteria. In a drop of kefir 8 days old, the lactic acid bacteria are very abundant and the yeast cells have entirely disappeared.

#### THE GINGER-BEER PLANT.

The following account of the ginger-beer plant, and the organisms composing it, is condensed from H. Marshall Ward's long paper in the Transactions of the Royal Society of London.

When seen in the fresh state, as it comes from flasks or other vessels, the ginger-beer plant presents the appearance of solid, white, semi-translucent, irregular, lumpy masses, not unlike pieces of soaked sago or tapioca; these lumps are brittle, like firm jelly, and their size varies from that of a pin's head, or smaller, to that of a large plum, or larger. Opacity and brittleness vary, even in the same lump. Fresh-dried lumps do not dissolve in water, even if boiled. When thoroughly dry they are often hard and horny. Fresh moist specimens are usually distinctly acid, though in varying degrees. The most striking characteristics of these lumps of ginger-beer plant become evident only when they are placed in saccharine solutions. After some days in a closed soda-water bottle three-fourths full of Pasteur's fluid, a lump of ginger, and a few lumps of the ginger-beer plant, kept in a warm place, the liquid is found to be very turbid and more or less viscous. The fermentation goes on rapidly. Much gas is produced and the container may explode if tightly closed. In time the viscosity increases, and it sometimes happens that the liquid becomes so thick that the gas-bubbles rise slowly. Viscosity is not due to the mere presence of yeast-cells, because they fall to the bottom, but to the presence of innumerable swollen or slimy vermiform bodies distributed through the mass of the liquor. Myriads of rod-shaped bodies (bacteria) are also observable. The increasing deposit below is also found, in later stages, to consist of bacteria, swarming amongst the yeast-cells. The "ginger-beer" is distinctly acid, as well as viscous.

As time goes on, the surface of the liquid usually becomes covered with a dense scum, unless very well corked and protected.

The problems then which present themselves are:

What is the yeast which so rapidly spreads in the earlier stages of fermentation?

What are the slimy vermiform bodies in the liquor?

What species of *Schizomyces* are present?

What does the scum consist of?, and finally,

What have all, or any, of these organisms to do with the ginger-beer plant, and the conversion of the saccharine liquor into "ginger-beer"?

In an effort to solve these problems, almost two thousand separate cultures, each extending over periods of from several days to months, and even in some cases to two years, were made. These cultures were of three kinds: (1) Large cultures in flasks, usually liquids, sometimes solid gelatin; (2) smaller cultures in tubes; and (3) cultures in hanging drops, made in sterilized cells under the microscope. Every piece of apparatus was heated in a hot-air chamber to at least 140° C. for 2 hours, and everything was lifted by forceps, similarly treated.

#### VARIOUS ORGANISMS FOUND IN THE GINGER-BEER PLANT.

It was apparent from the start that the ginger-beer plant is a body, consisting of several organisms, or, at least yielding more than one definite organism. Investigation has shown, however, that two specific cryptogams constitute the ginger-beer plant proper, and are necessary for its formation and peculiar action, while the rest are merely accessory or foreign organisms.

Of the two essential forms, one is a new species of *Saccharomyces*, the other a new and very remarkable species of *schizomyces*.

Of two non essential forms, found in all the specimens examined, one is a yeast-like form, *Mycoderma cerevisiae* Desm., while the other is the vinegar organism—*Bacterium aceti* Kütz.

The intruders most commonly met with are species of *Saccharomyces*, *Bacillus*, *Micrococcus*, *Oidium*, *Torula*, *Dematium*, and one or two ordinary mould fungi, of which *Penicillium* is by far the commonest.

The new yeast *Saccharomyces pyriformis* which resembles *S. ellipsoideus*, is the most important one met with in this investigation, being constant in every specimen examined, and undoubtedly the yeast principally concerned in fermentation of ginger-beer. It induces active fermentation in sugar-solutions, either cane-sugar, or glucose [no statement respecting lactose in this place, but elsewhere it is said that milk sugar can not take the place of cane sugar or glucose], resulting in a copious evolution of carbon dioxide gas, and in the formation at the bottom of the flasks, tubes, etc., of a voluminous white pasty deposit, consisting of characteristic colonies of budding-yeast cells. Pure cultures were readily obtained, both by the dilution method, and by growth on gelatin media; cultures were obtained from single cells grown in hanging drops. The single cell is globoid, or more commonly ellipsoid, or ovoid in shape, colorless and translucent, and measures from 6 to 7  $\mu$  long  $\times$  5.5  $\mu$  broad, though smaller and larger cells are found. The pyriform cells occur in the surface film. There is no limit to the size and shape of the colonies.

In very active, vigorous cultures of this yeast, the protoplasm gives a strikingly clear glycogen reaction—on adding iodine dissolved in an aqueous solution of potassic iodide, the cells turn dark sienna red, or red-brown. Ascospores occur repeatedly, and with singular distinctness. They are formed on moist gypsum blocks and also on the surface of gelatin.

This species is named *Saccharomyces pyriformis* from characteristic pear-shaped aërobian cells.

Chief characters: A low, or bottom-fermentation yeast, which inverts and ferments cane-sugar. Ordinary cells ovoid or globose, ranging from 5 to 9 $\mu$  in diameter, though smaller and larger ones occur. Ascospores formed in from 2 to 4 days, at 25° C. and lower. Aerobian forms, as films, of pyriform, or sausage-shaped cells are developed in wort in 21 days. Occurs in "home-brewed ginger-beer," and is the predominant form in the so-called "Ginger-beer plant."

Whenever the fermentations were carried on or finished with access of air, a dense wrinkled skin formed at the surface; and, since this occurred when the air had to filter through sterilized plugs of cotton wool, there can be no doubt as to the origin of the fungus from the inoculating material. This growth was identified as the very polymorphic *Myoderma cerevisiae* of Desmazières. It is not a true *Saccharomyces* for it does not form ascospores, and differs in several respects from the true yeasts, in the narrow sense. It is distinctly an aerobian form; is unable to invert cane-sugar or to bring about its fermentation; it is apt to appear on lager beer even in cold cellars; cells are elongated, less translucent than active *Saccharomyces* cells; average size, 6 to 8 $\mu$  long, by 2 to 4 $\mu$  broad.

A pink or rosy yeast *Cryptococcus glutinis* Fres. ?, also occurred. This organism is not a necessary part of the ginger-beer plant, and is not always present in cultures of this "plant."

A small yeast, the cells of which are very nearly spherical, averaging about 2.3 to 3.7 $\mu$  in diameter (*Yeast D*) was frequently met with, but is not a normal constituent of the ginger-beer plant. Not sufficient knowledge of its characters to warrant any specific name.

The ordinary beer-yeast (*Saccharomyces cerevisiae*) and two other forms, not identified with certainty, one of which is probably *S. apiculatus*, were found occasionally. These are not concerned in the formation of the ginger-beer plant.

The new schizomycete called *Bacterium vermiforme* is a peculiarly vermiform organism, enclosed in hyalin, swollen, gelatinous sheaths, and imprisoning the yeast-cells of *Saccharomyces pyriformis*, etc., in the brain-like masses formed by its convolutions. It is a constant and essential form—essential because the ginger-beer plant can not exist as such without it. It is the swollen sheaths of this organism which constitute the jelly-like matrix of the "plant."

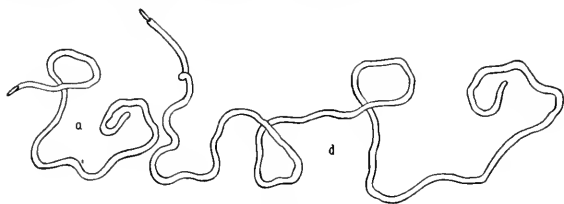


Fig. 41.\*

Various attempts were made to isolate this organism—a difficult thing to do at first, because then it was not known what relation the naked forms bore to the sheathed cocci, rodlets, filaments, etc. Two distinct phases of this organism exist—the vermiform, sheathed stage found in acid saccharine media saturated with carbon dioxide, and the motile naked filaments, rodlets and cocci met with in neutral bouillon and other incomplete nutritive media containing oxygen. It was necessary to take the precaution of cultivating both forms side by side, under exactly similar conditions, varied one by one similarly for each. In the presence of oxygen the bacteria promptly escape from their sheaths. If these unsheathed rods are grown in the presence of yeasts, *i.e.*, carbon dioxide producers and oxygen consumers, the sheaths form again.

When a small piece of the gelatinous form, *i.e.*, the compacted coils of sheathed filaments, rodlets, etc., of *Bact. vermiforme* is put into a test-tube of suitable nutritive fluid (*e.g.*, Pasteur-bouillon, beet-solution, bouillon +5 per cent of sugar, etc.) and kept at 15° to 18° C., the usual course of events is as follows:

The liquid becomes more and more turbid after 48 hours or so; then a whitish film begins to form above, and a deposit at the edges of the level of the liquid, while a similarly whitish, granular or cloudy looking deposit falls to the bottom. In from 7 to 14 days the rapidly increasing deposit becomes more and more gelatinous, and at length assumes the consistency of a sort of jelly. This gelatinous cushion at the base consists of the sheathed coils so often referred to; the film and ring at the level of the liquid, and the turbidity throughout the body of the same, are chiefly due to the free filaments and rodlets already described as escaping from the sheaths. The preliminary turbidity of the liquid is due to the motile forms of these filaments and rodlets. Flagella could not be demonstrated. The sheaths may grow end on (fig. 41) or sidewise (fig. 42).

\*FIG. 41.—Two stages of Marshall Ward's *Bacterium vermiforme* in a hanging drop of Pasteur bouillon stiffened with gelatin. I have omitted intermediate stages *b* and *c*. The stage *d* was drawn 21 hours later than *a*. After Ward.



"It must, therefore, be concluded that this schizomycete is able to live and grow in an acid saccharine solution, with suitable minerals and nitrogenous materials, not only in an atmosphere totally deprived of oxygen, but in one of vapor which is so attenuated that it is practically a vacuum so far as permanent gases are concerned—and that only forms its gelatinous sheaths if carbon dioxide is present." (See figs. 43 and 44.)

A *ginger-bacillus* (*Schizomycete* No. 2.) is frequently met with in fermentations to which lumps of unsterilized ginger were added, but is not essential to the formation of the ginger-beer plant. It occurs on ginger rhizomes.

Cultures of *Schizomycete* No. 3 (*Bacterium aceti* Kütz.), demonstrated not only that this bacterium is not a normal or necessary constituent of the ginger-beer plant, but also that it can not be induced to form a submerged commensal growth with any of the yeasts.

#### SYNTHESIS OF THE GINGER-BEER PLANT FROM PURE CULTURES.

The most conclusive proof of the accuracy of the foregoing studies is afforded by the re-constitution of the ginger-beer plant as such, by bringing together pure cultures of the organisms composing it, and showing that the specimens so produced act like the original specimens. The other forms mentioned above were tried in various combinations, but only the two essential ones, *Saccharomyces pyriformis* and *Bacterium vermiforme* were successful. The relations between this yeast

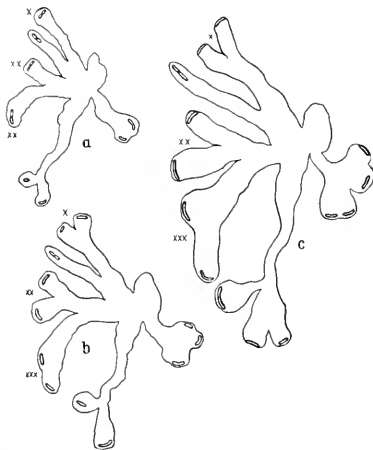


Fig. 42.\*

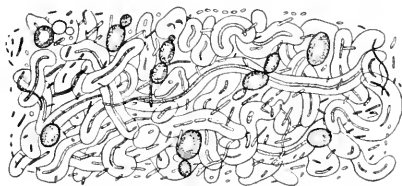


Fig. 43.†

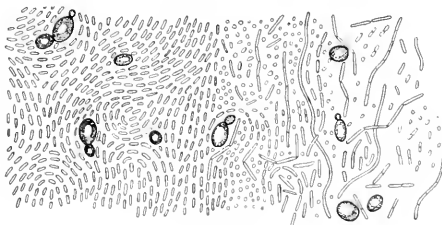


Fig. 44.‡

and bacterium are those of true symbiosis. The ginger-beer plant forms only in acid media and in closed vessels, or in other conditions in which the free oxygen has been removed and carbon dioxide substituted, e.g., under *Mycoderma pellicles*. The nutritive substance must also contain carbohydrates. Cane-sugar is better than glucose; milk-sugar will not do; sterilized ground rice may be used in place of ginger—the starch in the ginger being apparently the essential. The schizomycete will grow in the absence of these substances but no sheaths form.

The origin of the ginger-beer plant is involved in obscurity, but there is evidence to show that the yeast is introduced from the grocer's shops attached to the ginger and brown sugar employed in ordinary practice, while the bacterium is introduced with the ginger.

\*Fig. 42.—Splitting of sheaths in Marshall Ward's *Bacterium vermiforme*: a, condition when fixed under the microscope in a drop of ginger-gelatin; b, 6 hours later; c, 18 hours after b. The corresponding parts are designated by x's. After Ward.

†Fig. 43.—The ginger-beer plant, a compact mass due to growth of *Bacterium vermiforme* with yeast in a suitable saccharine medium. This figure shows appearance after 15 days in Pasteur-bouillon. "The filaments and rodlets ensheath themselves as soon as the carbon dioxide is in excess, and entangle the well-developed yeast-cells in the coils of the gelatinous matrix. The mass becomes denser and denser, and at last forms the hard, brain-like lumps of the ginger-beer plants." After Ward.

‡Fig. 44.—*Bacterium vermiforme* and *Saccharomyces pyriformis* grown in an unsuitable medium in which no sheaths appear on bacteria and no symbiosis takes place. Drawing made after 15 days in ordinary bouillon. "The yeast buds slowly and for a short time only. The Schizomycete grows out into filaments which rapidly break up into very short rodlets (bacteria) and cocci." After Ward.

In addition to large quantities of carbon dioxide, ginger beer contains during early stages of fermentation traces of alcohol and acetic acid, while relatively large quantities of an acid resembling lactic acid, if not identical with it are formed.

Just what each organism gains from the combination was not made out clearly, but the yeast seems to do better in the presence of the bacterium than where separated from it.

The following pertinent paragraphs from Ward's paper may close this review:

"Everything points to the view that the relations between the yeast and the bacterium are those of true symbiosis, because every attempt to feed the schizomycete with dead yeast-cells or decoctions of such, or detect it embracing such cells in a dead or feeble condition has failed.

"It is significant that the synthesis of this dual organism—which is so strikingly like the lichen that we may compare it forthwith with one of the gelatinous forms—was most easily brought about by adding the yeast-cells to already advanced cultures of the bacterium, both having been grown in the same medium and under like conditions. \* \* \*

"The schizomycete is favored by obtaining some substance or substances directly they leave the sphere of metabolic activity of the yeast-cells; it can benefit by the presence of these substances, even apart from the living yeast, though to a less extent.

"The yeast, on the other hand, benefits by these substances being removed and destroyed, hence its renewed and continued activity—as evidenced by the steady and copious evolution of carbon-dioxide for weeks, and the corresponding increase of the yeast-cells by budding—when the symbiosis is established.

"For the present this can only be regarded as a hypothesis. It might be objected that I have inverted the order of things—that, since the schizomycete is able to evolve small quantities of carbon dioxide daily from saccharine solutions, it may be that its powers are enhanced by the yeast removing inhibiting substances of its activity. The objection is possibly valid, but I think the former hypothesis explains most of the facts: How, for instance, is it to be explained that the schizomycete slowly and steadily converts the whole of the liquid sugar-solution into a solid gelatinous mass, if the organism excretes such inhibiting substances?"

#### THE SO-CALLED "BEER-SEED."

The writer has had but one opportunity of seeing kefir-like grains. These were sent from Missouri to the Department of Agriculture under the name of "California Beer-seed." They were acid to litmus paper, and had a feebly acid, rather agreeable, ester-like odor. The roundish gelatinous granules (fig. 45) consisted of several

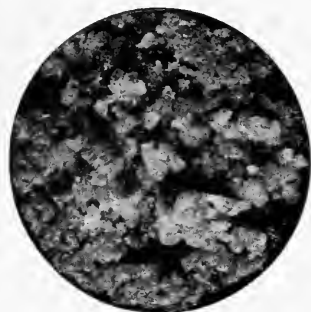


Fig. 45.\*

kinds of bacteria, and of one or more kinds of yeasts, together with oblong, large, jointed threads which stained like yeast and were interpreted as *Oidium lactis*. The majority of the yeast-cells were round or roundish and the long oval or elliptical ones appeared to be of a different sort. Morphologically there appeared to be at least three kinds of bacteria in the grains, but no coccus forms were detected. Quite unlike the kefir figured by Beyerinck, the yeast-cells were not in a uniform thin layer on the surface, but were distributed through the grains in little clumps, most of the clumps, however, being in the outer parts of the grains (fig. 46). The greater portion of the bacterial mass consisted of rather thick short threads. Long filaments were exceptional. The appearance of the more common yeast and schizomycete as crushed out in water is shown in figure 47. The general relation of yeasts and bacteria in the substance as determined by thin sections of fresh grains and by microtome sections of embedded material is shown in fig. 48. The writer did not observe any bacterial forms corresponding to the end-splitting ones figured and described by Marshall Ward, but prolonged study was not given to the subject.

Poured-plates made from the granules on ordinary +15 beef-bouillon agar yielded a considerable number of small, slow-growing white colonies which proved to be a yeast.

\*FIG. 45.—Grains of "California beer-seed." Received in 1908 from Missuri.

No bacteria were obtained on these plates. This yeast fermented grape-sugar in peptone water readily and also cane-sugar and maltose, but not lactose.

This yeast grew on potato in a thick whitish layer. It grew readily in the thermostat



Fig. 46.\*

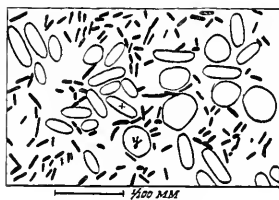


Fig. 48.†

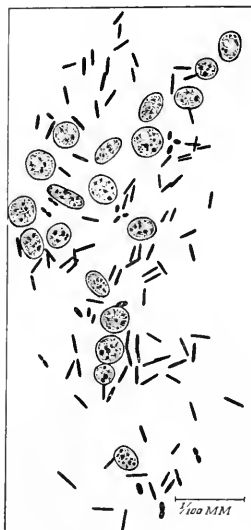


Fig. 47.‡

at 28° C. On agar streaks it sporulated freely after some days, the protoplasm, rounding off into two or more parts. Often three spore bodies and sometimes four or more in a cell were observed (fig. 49). The appearance and behavior of this yeast suggested *Saccharomyces cerevisiae*.

Sterile litmus milk inoculated by putting some of the grains into it did not redden, did not coagulate at once and did not produce any gas-bubbles. Gradually the litmus was reduced and remained reduced for a very long time. The milk also finally curdled. It was blue after the reduction ceased.

It would appear, therefore, that some kefir-like grains contain a yeast incapable of fermenting lactose, while grains from other sources contain a yeast capable of fermenting lactose. The relation of kefir to the ginger-beer fermentation remains to be determined.

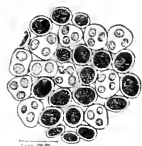


Fig. 49.§

\*FIG. 46.—Free-hand unstained section through one of the "beer-seeds," surface of grain being at left. The uniform gray structure represents bacterial zoogloae, the darker masses the undisturbed position of imprisoned clumps of yeast.

†FIG. 47.—A small portion of "beer-seed" crushed in water and drawn after staining with carbol fuchsin. The yeasts buried in the zoogloae were not budding, most of them were globose or nearly so.

‡FIG. 48.—Another crushed-out water mount of "beer-seed," showing both round and elongate yeasts, the contents omitted. x and y stained alike with fuchsin and both forms were full of coarse granules.

§FIG. 49.—Spores or spore-like bodies in yeasts on agar sub-culture (streak) made from an agar-plate poured from crushed "beer-seed." Age 16 days. Stain, carbol fuchsin.

## BACTERIA WITH FUNGI.

In 1903, Zederbauer published a paper on the Myxobacteriaceae, in which he claims that he has demonstrated that certain of these organisms are a symbiotic combination of bacteria and fungi.

His first investigations were made on a reddish form, which he calls *Myxococcus incrusters*, growing on sponges used for wetting gummied paper. This form, which looked like a slime mold was made up of bacteria, chiefly, with occasional fungous hyphae, and chains of small round bodies which he thinks were conidia.

He succeeded in obtaining pure cultures of the bacteria (some of them, at least) on peptone gelatin. At room-temperature, in the light, they grew rapidly, forming a film of branching radiating chains of bacteria on the surface. In rather old colonies these floated for a time on the liquefied gelatin, then sank to the bottom. Growth occurred also below the surface, but was not so luxuriant.

All cultures, in repeated experiments, produced the same bacterium. The color of the *Myxococcus* is not shared by the bacteria. The colonies are dirty white. Spores were formed in old cultures. No cilia were demonstrated, nor was the movement like that of ciliated forms. On agar, growth appeared similar to that on gelatin, but the agar was not liquefied. The bacteria did not grow on sterilized bread or potato.

The fungus was also cultivated separately. Spores taken from the so-called *Myxococcus* germinated in the usual manner, forming several celled hyphae, which soon produced conidia, like the original spores. Mycelium also developed from oidia which were formed by the breaking up of filaments.

Although Zederbauer thus cultivated both fungus and bacteria separately, he did not succeed in reproducing the original mixture, the *Myxococcus*, by growing them together.

Cysts, he states, like those described by Thaxter, were found. These were composed of bacteria and chains of conidia surrounded by a common envelope, probably composed of hardened slime secreted by the bacteria. On germination, this bursts and the new organism begins growth.

The color of his *Myxococcus* is not constant. It may be red, pale yellow, and sometimes black.

A form which he calls *Chondromyces glomeratus* was found in several localities growing in groups upon the cut surface of beech stumps which had not begun to decay. The slimy red outgrowths 4 to 5 mm. high, resemble Tremella. This form he says was also composed of bacteria and fungous hyphae. The long slender hyphae, rising from spores at the base, intertwined and formed at the surface a thicker layer of conidiophores bearing chains of conidia. The very small rod-like bacteria which swarmed in the interior were actively motile.

Several conidia were germinated in a moist room, forming hyphae, which, however, did not produce conidiophores.

The bacteria, which stained with methylene blue and fuchsin, grew on gelatin and agar. On gelatin plate cultures at room temperature or in the thermostat at 20° C., small dirty white drops were formed which united and liquefied the gelatin in hollows. In cultures kept near the window, growth was strongest near the light. Streak cultures behaved very much like plate cultures. The bacteria did not liquefy agar but formed over the whole surface a dirty white layer, starting from small round flecks. All cultures were fluorescent.

The flagella, attached to all parts of the body, were stained with Van Ermengem's stain as modified by Hinterberger. In some cases they were ten times the length of the bacteria. Spore formation was not observed.

In gelatin cultures when hyphae were brought in at the point of inoculation they took on irregular shapes and seemed to form conidia. Chlamydospores were also formed in such cultures.

Zederbauer claims that the origin of these demonstrates that they are fungous spores and not bacteria as claimed by Thaxter.

The first part of this paper is devoted to speculations on whether Thaxter's statements and figures can be interpreted as indicating the presence of mycelium in the Myxobacteriaceae. There seem to be a good many uncertainties connected with his own researches.

Dr. Thaxter's comment on this paper is as follows:

"This treatment of the group, though novel, seems somewhat hasty; especially in view of the fact that the figures and descriptions given in this paper show very clearly that its author is as yet unacquainted with any member of the order he discusses, having been misled by fancied resemblances and influenced no doubt by an exaggerated notion of the difficulties associated with the differentiation of rod-like bacteria from *Oedoecephalum*, *Torula*, and similar hyphomycetous types. A specimen

of *Myxococcus incrustans* (*Torula myxococci-incrustantis* n. sp.  $\times$  *Bacterium myxococci-incrustantis* n. sp.), which Dr. Zederbauer has kindly communicated to the writer, serves further to confirm this impression. An examination of this specimen shows it to consist of a horny incrustation which at least closely resembles a dried up mouldy plasmodium, blackened by the abundant fructifications of a toruloid hyphomycete; and from the fact that the bulk of the mass consists of calcic carbonate, one might perhaps venture the suggestion that it may be related to the Physaraceae. That a number of organisms are associated in this lichen can scarcely be disputed; yet whatever it may prove to be, either as a whole, or in detail, it surely has no connection with any of the Myxobacteriaceae, as this group is at present understood. \* \* \*

"Since the present paper was sent to the Gazette for publication a mounted preparation containing sections of authentic material of *Chondromyces glomeratus* has been received from Dr. Zederbauer and proves to be the conidial condition of *Coryne sarcoides* (Jacq.) Tul., to which the name *Tremella sarcoides* was given by Fries. It need hardly be remarked that this fungus is a widely distributed and very common form, well known to mycologists, having no connection either with 'lichens' or Myxobacteriaceae."

In 1903, Molliard succeeded in obtaining normal perithecia of *Ascobolus* in cultures on nutrient media by introducing into such cultures certain bacteria. The following is a brief account of his work.

He easily obtained the germination of ascospores of *Ascobolus* gathered aseptically. A luxuriant mycelium was formed with abundant arthrospores, like those found by Brefeld. The white mycelium growing from these filled the culture-tube but produced very few perithecia. These appeared only after 4 to 6 weeks and did not mature. In some other cultures perithecia appeared after 10 to 15 days and matured normally. In such cultures the perithecia arose on a few filaments, rising into the air above the rest, which were wet with the liquid which bathed the substratum. A microscopic examination showed that these cultures were contaminated with bacteria. As the original ascospores were taken from colonies on cowdung, Molliard assumed that the bacteria were carried over from that source. To test this assumption he cultivated *Ascobolus* on sterilized cowdung. A vigorous mycelium was produced which remained indefinitely sterile, i.e., did not produce perithecia. When, however, the mycelium was cultivated on sterile cowdung and then contaminated with bacteria isolated from the tube cultures, only a few filaments appeared on the exterior of the substratum, but these produced, in the course of 20 days, numerous large perithecia.

From these results Molliard concludes that the bacteria are in some way responsible for the formation of perithecia, and that we have thus presented a method for obtaining perfect forms of many coprophilous and humicolous fungi.

#### BACTERIA WITH MYXOMYCETES.

Pinoy speaks of a symbiosis with bacteria as necessary for obtaining cultures of myxomycetes. In his first paper he says that various workers have had bacterial contamination in their myxomycete cultures and raises the question: "Can one obtain a pure culture of myxomycetes?" He obtained cultures of two species of myxomycetes with bacteria on solid media. The bacterial species was identified as *Bacillus luteus* Flügge. The myxomycetes were *Chondrioderma difforme* and *Didymium effusum*. The only evidence given that bacteria are necessary to the growth of these myxomycetes is that, when transfers of spore material were made after flaming the sporophores, some tubes remained sterile and others gave mixed growths.

According to Potts there is no symbiosis. The *Dicty. mucoroides* profits by the presence of the bacteria, but the latter grow equally well when the myxomycete is absent. When the *Dictyostelium* fruits on bacterial colonies it causes them to become transparent. A large proportion of the bacteria forming such colonies are dead (98 per cent in one case, 83 per cent in another), and when such colonies are examined under the microscope they are seen to contain many involution forms and remnants of bacteria, but few or no normal bacteria. The bacteria are killed and then consumed.

*Dictyostelium* was cultivated in combination with four different bacteria: *Bact. fimbriatum*, *Bacillus megaterium*, *Bacillus subtilis*, *Bact. fluorescens liquefaciens*. It can

not make use of the by-products of these bacteria, but nourishes itself at the expense of the bacteria themselves.

"Die enorme Zahl von Bacterien, die *Dictyostelium mucoroides* verdaute, und seine geringen Anforderungen an die Ernährung erklären es, dass Bacterien den Hauptbestandtheil seiner Nahrung bilden und für ein mässiges, wenn nicht reichliches Wachstum genügen."

In a second paper Pinoy states that he freed spores of *Dictyostelium mucoroides* from the presence of *Bacillus fluorescens liquefaciens* by exposing them to a temperature of 50° for one hour. Under these conditions the bacteria were killed but the spores of the myxomycetes were not killed. Such spores germinated readily in the presence of various bacteria e. g., *Bacillus fluorescens liquefaciens*, *Microbacillus prodigosus*, *Bacillus coli*, etc. They did not germinate when sown by themselves. The development of the myxomycetes was more or less abundant according to the bacterial species used in connection with it. When fluorescent bacteria were used the *Dictyostelium* became a yellowish color. On the contrary when the *Bacillus prodigosus* was used the spore-heads were white with a very slight tint of rose color.

Vuillemin has also published a note on this subject. He states that he cultivated *Dictyostelium mucoroides* in test-tubes, cotton plugged, on agar containing 0.5 per cent peptone and 0.2 per cent maltose, at laboratory temperature, protected from the light. The sowings were made from white spore heads and these often contained bacteria which he states to have been a fetid fluorescent species. All the tubes which showed any growth of *Dictyostelium* also contained the bacteria. The fruiting pedicles appeared the third day. If the sowings had not developed any bacteria there was no visible growth of the myxomycetes, although the microscope showed amoeba-formed bodies to have issued from the spores. To obtain growths of the myxomycetes from these apparently sterile tubes it was only necessary to introduce a culture of the bacillus. When pyocyanic bacteria were substituted the results were negative.

Nadson believed that the bacteria rendered service by producing an alkaline substratum. Vuillemin states that the bacteria do not act indirectly, by modifying the substratum, but that they serve directly as food for the amoeboid bodies of the myxomycetes.

Nadson, whose paper is earlier than any of those already referred to (1899), states that he obtained absolutely pure cultures of *Dictyostelium mucoroides*, but that these were weak dwarfed forms, giving generally no proper conception of the species. He also speaks of a symbiosis and says that the ordinary companion of this myxomycete is *Bacillus fluorescens liquefaciens* Flüggé.

In 1905 Pinoy published a paper on the rôle of bacteria in the development of *Plasmodiophora brassicae*, the myxomycete occurring in hernia of the cabbage.

He found in pieces of young tumors of cabbage obtained by experimental infection, that some cells invaded by the parasite contained also masses of bacteria (forms of coccobacillus occurring either singly or in pairs).

He followed this microscopic work by cultures as follows:

The surface of large tumors, showing no trace of decay was burned deeply with a hot iron, and portions removed by means of flamed pipettes. The spores of the parasite contained in great numbers in this material were sowed on the ordinary media and produced numerous colonies of bacteria. He thinks, therefore, that the bacteria were introduced into the root of the cabbage by the parasite. What rôle do they play?

Pieces of healthy young turnip were removed aseptically by means of a sterile punch (Borrel's), placed in flamed tubes and sowed with the spores of the fungus. The tubes were then sealed in the flame and placed in the thermostat at 22° C. During the first days scattered colonies of aerobic bacteria arose, which, however, ceased to grow when the oxygen was exhausted. Five days after sowing, the cells of the turnip contained *Plasmodiophora* in various stages. Many cells were filled with spores. When the same experiment was carried on in tubes plugged with cotton, i.e., exposed to the air, the aerobic bacteria which accompanied the spores developed more abundantly and brought about the decay of the turnip. When anaerobic bacteria were accidentally introduced the growth of the myxomycete was stopped.

The presence of aerobic bacteria seems to be necessary to the life of the myxomycete outside of the host cells. Thus among the great number of tubes of agar sowed with the spores, the greater part of those containing bacteria gave at once a development of the fungus (formation of the amoeboid individuals which soon perished) while in two containing no bacteria, the spores, though perfectly preserved did not germinate.

Pinoy thinks, therefore, that the bacteria introduced with the parasite contribute to the decay of the tumor of the cabbage when the conditions are favorable to their multiplication.

Pinoy continued his interesting studies, publishing a monograph in 1907 wherein he considered at length the relations of various bacteria to several species of Myxomycetes in the Group Acrasidae; then to several species of the endospore-bearing forms, and finally to *Plasmodiophora brassicae*. I summarize as follows:

Pinoy, like Nadson, found *Bacterium fluorescens liquefaciens* associated with *Dictyostelium mucoroides*.

On agar poured plates the spores of this slime mold germinate only in the presence of bacterial colonies. Elsewhere the spores do not germinate. But those spores which do not germinate can not for that reason be assumed to be bacteria-free. They also bear bacteria which can be resuscitated by putting them into bouillon, which clouds after 6 to 8 days. Nadson, Potts, and Vuillemin did not take into account these tardily developing bacteria.

*B. fluorescens* is killed by exposure to 50° C. for 1 hour. On the contrary, 80 per cent of the spores of *Dictyostelium mucoroides* are still able to germinate after such an exposure. It is possible also to purify the spores by heating them for 2 minutes at 56° C. Spores about 8 days old are most resistant. Spores thus purified will never germinate on any culture medium whatsoever, unless suitable bacteria are added.

Potts' statement that he was able to grow *Dictyostelium mucoroides* on the dead bodies of his *Bacterium fimbriatum* is regarded as illusory, i. e., the purity of his spores is questioned. Pinoy showed the need of living bacteria quite clearly as follows: Into a flask of culture medium he plunged a collodion sack attached to a projecting tube. After proper sterilization, the medium inside the collodion sack was inoculated with purified spores of *D. mucoroides*, that outside with *Bact. fluorescens*. The spores of the Myxomycete germinated but the amoebae soon rounded off and died. The dead bodies of *Bact. fluorescens* (killed by heat, ether and chloroform) were also placed in the collodion sack without result.

"En résumé, le *D. mucoroides* ne peut vivre qu'en association avec une Bactérie vivante. Toutes les Bactéries ne conviennent pas également."

The culture medium exerts a marked influence, e. g., on potato sowed with mixtures of *Dicty. mucoroides* and *Bact. fluorescens* the slime mold does not develop. The same is true on this medium whatever bacterium is used. With most bacteria this is also true on peptone agar, or meat broth agar. The best culture medium found was flax-seed agar. On this the bacteria grow abundantly and the harvest of *Dictyostelium mucoroides* reaches its maximum.

Bacteria that stain by Gram are not suitable for such cultures. In general, bacteria which do not stain by Gram allow the *Dictyostelium mucoroides* to grow. The *Dicty. mucoroides* will not grow in the presence of pure cultures of *Bacillus megaterium*, but if this organism is added to *Dictyostelium mucoroides* with *Bact. fluorescens* growth may be had on beef agar.

The growth of the *Dictyostelium mucoroides* may be regarded as a parasitism on the bacteria. They are absorbed into the vacuoles and digested. Pinoy confirms Metchnikoff's observation that the liquid in the vacuoles is acid.

Neutral red is recommended as a stain for the bacteria in process of digestion. It does not stain the living bacteria nor kill the Myxomycete unless too strong. Vesuvium may also be used. For details see paper. Potts did not find bacteria in the interior of the vacuoles of *Dicty. mucoroides* because his technic of fixation and staining was insufficient, he therefore formed the erroneous hypothesis of an extra-cellular diastase.

Grown with *Bacillus coli*, the enzyme isolated at the end of 40 hours liquefied gelatin. It acts in neutral or feebly alkaline liquids. The acidity of methyl orange inhibits. It is therefore related to trypsin rather than to pepsin. It is destroyed at 55° C. Its maximum of activity is about 38° C.

It has scarcely any action on fibrin or on albumen coagulated by heat.

"Acradiastase" does not act on bacteria killed by heat, but readily dissolves those killed by ether or chloroform.

"The best bacterial test is also *B. coli* which is not self-autolytic, and a chloroformed emulsion of which remains cloudy.

"Let us take such an emulsion and add some drops of it to two tubes: one containing the normal diastase liquid, the other the same quantity of this liquid boiled. They are put into the thermostat at 38°. Of these two tubes, equally cloudy, the check after some hours remains cloudy, while the other has become almost completely transparent."

There is no precipitate and therefore the clearing can not be ascribed to agglutination.

"Ainsi *Dictyostelium mucoroides* ne peut se développer qu'avec des Bactéries; il est parasite des colonies bactériennes; ses myxamibes ingèrent les Bactéries et les digèrent dans leurs vacuoles à l'aide d'une diastase dont l'action est assez semblable à celle de l'amibodiastase."

Similar results were obtained with other species, *i. e.*, *Dicty. purpureum*, and *Polysphondylium violaceum*, showing that these also are bacterial parasites.

These Acrasieae are strictly aerobic. As soon as a tube is sealed growth ceases. The amount of humidity greatly influences the morphology of the sporophores. The optimum temperature for growth is between 22° and 25°. Above 28° there is no development. They will grow at a temperature as low as 8° but then very slowly.

The morphology and the color of the Myxomycete are both changed by changes in the substratum, *e. g.*, if *Bacillus subtilis* is added to mixed cultures of *Dictyostelium mucoroides* and *Bact. fluorescens*, the sporophores are longer and branched forms are frequent.

Under some circumstances bacterial pigments are absorbed by the living Myxomycetes. The author holds that certain Acrasieae described as distinct from *Dicty. mucoroides* on account of their variation in color are only the same species associated with different chromogenic bacteria. These bacterial pigments therefore have a taxonomic importance in the Acrasieae. Grown with *Bact. fluorescens* the young fructifications of *Dicty. mucoroides* are fluorescent and the old ones are color of a dead leaf; grown with *B. coli* the fructifications of this species are pure white and remain so. When *Polysphondylium violaceum*, which has a pigment of its own, is grown in the presence of *Bacterium violaceum* its color becomes paler, the pigments of the two being chemically dissimilar bodies.

Similar results as regards necessity for living bacteria were obtained with *Didymium difforme* and *D. effusum*.

So far as the writer has observed bacteria always occur in the club-root of crucifers along with *Plasmodiophora brassicae*.

#### ONE BACTERIUM WITH ANOTHER.

This subject is a very large one and no attempt has been made to cover it either in the text or bibliography.

According to Beyerinck and Van Delden their *Chroococcum* assimilates nitrogen only when it enters into symbiosis with other bacteria—*Granulobacter*, *Aerobacter*, etc.

In 1906, Keding published his Weitere Untersuchungen. He found *Azotobacter* not only on the surface of *Fucus* and several other salt water algæ, but in dune sand near the roots of strand plants, and in all investigated soils, except moor soil. *Azotobacter* is able, he says, to assimilate the nitrogen of the air in pure culture, and this ability was not increased by growing it in combination with other bacteria. The sea forms of the organism can grow in the presence of 8 per cent salt.

According to Bottomley "*Pseudomonas radicola* and *Azotobacter*, together make a powerful combination for the fixation of free nitrogen." These are both said to have been isolated from the algal zone of the root-tubercles of eycads. He inoculated oats, barley, hyacinths (*galtonia*), and parsnips with mixed cultures. Best results with oats which were nearly doubled in weight.

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ARE ANY BACTERIA KNOWN TO CAUSE DISEASE IN BOTH PLANTS AND ANIMALS?  
EVIDENCE FROM INOCULATING PLANT PARASITES INTO ANIMALS—EVIDENCE  
FROM INOCULATING ANIMAL PARASITES INTO PLANTS—DO PLANTS HARBOR  
ANIMAL PARASITES?

Theoretically, this subject is of great importance. Actually, very little of positive value has been developed by the studies thus far undertaken, *i. e.*, the results in general have been negative. Most bacterial plant parasites are unable to grow at blood-heat, and for this reason may be regarded as harmless to man and the domestic animals.

Most animal parasites are more or less delicately balanced to the conditions prevalent in animal bodies and not to those occurring in plants, although when inoculated into certain plants some of them have remained alive in the vicinity of the wound for a considerable period.

The chief danger to health would appear to lie in the ingestion of plants whose surfaces have been contaminated by animal pathogenic organisms, *i. e.*, in the use of raw vegetables and salad plants, particularly those grown on lands fertilized with untreated sewage. Sewage should be sterilized before it is passed into streams or flooded upon agricultural lands. Vegetables grown on lands manured with night-soil or with untreated sewage should not be eaten raw. It would be entirely proper to prohibit altogether the sale of such vegetables.

The principal studies, so far as known to the writer, are summarized in the following paragraphs.

ANIMAL PARASITES INOCULATED INTO PLANTS.

Grancher and Deschamps (1889) experimented on seedling radishes and carrots grown in special boxes and watered repeatedly with typhoid cultures diluted in water (20 cultures in 10 liters of sterilized water). The experiment was begun April 9 and finished June 6. Nine gelatin plates were poured from the inner tissues with negative results, the plants being wiped and flamed, and the pulp removed under sterile conditions.

Tests were also made by them of radishes and carrots from the garden of the hospital and of radishes, carrots, and asparagus from the municipal garden at Gennevilliers, 46 tubes of peptone-gelatin and 20 flasks of bouillon being inoculated. Part of these cultures were kept in the thermostat and the rest held at room temperature. All were negative.

Conclusion: *Le Bacille typhique et les microbes communs du sol ne pénètrent pas dans la pulpe des légumes sains.*

One of the hospital radishes yielded a common organism but its pulp was probably already invaded through a scratch on its surface.

In 1890 Loninsky\* published his paper in the Russian Medical Journal *Wratch*. It is believed that a rather full account of this paper will be welcome to English readers.

The author approaches this problem from the standpoint of a physician. If plants are capable of nourishing a single organism causing animal disease, to know it is a matter of great importance. It has long been known that disease-producing microbes can grow on dead vegetable matter, especially some culture media, *e.g.*, cooked potato. Whether they will grow on living plants is quite another matter. Up to this time living vegetables have been considered very unfavorable media for the growth of bacteria. The experiments of Buchner, Lehmann, Fernbach, Miquel and Grancher lead to one conclusion, *viz.*, that vegetables, seeds and plants do not contain microbes. "And, therefore," says the author, "I had in view to investigate whether the animal-pathogenic bacteria are able to find in the tissue of a living and growing plant a favorable soil for their existence."

\*Spelled also Lomnitzky, Lominskago, Lomnitzky, etc.

The paper is stated to be a preliminary one. For his experiments the author took three bacteria, viz, the bacillus of the Siberian plague, the bacillus of typhoid fever, and *Bacillus prodigiosus*. The plants inoculated were *Triticum vulgare*, *Agapanthus*, *Polygonum fagopyrum*, *Trifolium pratense*, *Sambucus*, *Hyacinthus*, and *Tulipa*. Heinz's results with his *B. hyacinthi-septicus* were known to Lominsky.

Two ways were chosen for investigating this subject:

(1) Seeds were planted on infected soil; (2) plants were inoculated by puncture, especially on the leaves. The surfaces of the seeds were sterilized by washing in soap and water and then in mercuric chloride 1:1000. They were afterwards left for half an hour in 1:5000 HgCl<sub>2</sub> or for one hour in 1:10,000 HgCl<sub>2</sub>. Seeds of wheat thus sterilized on their surfaces were treated in two ways. In the one case, they were plunged into colonies of the above named bacteria, then laid in a tin box on sterilized soil, and covered with about an inch of soil. This little box was then put on a glass plate and covered with a bell-jar, the upper opening in which was covered with cotton. From time to time sterile water was added. The seeds germinated in 5 to 30 days, the room temperature being 25° to 27° Celsius. In the other case, the germinations were sometimes made on moist sterilized cotton, but more often on boiled potatoes prepared as for bacterial cultures, except that to them was added a little of the following solution: water, 1,000; potassium nitrate, 1; potassium sulphate, 0.25; monopotassium phosphate, 0.25; magnesium sulphate, 0.25. A little ferrum phosphate in powder was also added. After sterilizing this medium the wheat was put in. This method enables one to decide whether the seed and substratum have been properly sterilized. Then, after a few days, the microbes were introduced on the end of a platinum needle.

The inoculations on the leaves were made into very young plants and into older ones. The very young plants were germinated in soil or in cotton, and when the green parts had reached a height of 2 to 5 cm. the specified microbes were inoculated by means of a needle. On adult plants the inoculations were made with a platinum needle shoved flat-wise between the upper and lower surface of the blade, after first washing the leaves in 1:1000 HgCl<sub>2</sub> and drying them in sterilized cotton. The surface of the wound was covered with collodion. Leaves of plants were also dipped into sterilized water to which the microbes had been added. After 3 to 42 days the inoculated leaves were examined microscopically. Cultures were also made from them and inoculated into animals. Leaves to be examined were hardened in alcohol. Bacteria in the growing tissues were stained by the methods in use for animal tissues. Three hundred experiments were made. The author's conclusions are as follows:

- (1) Disease creating microbes [animal pathogenic bacteria] may find conditions for their existence in tissues of the higher plants.
- (2) The uninjured cuticle prevents the entrance of bacteria.
- (3) Mechanical injuries of the leaves and stems of growing plants afford an opportunity for the entrance of bacteria into the tissues.
- (4) The bacillus of Siberian plague [*planobacter anthracis*], the bacillus of typhoid fever, and the *B. prodigiosus* can multiply and form colonies inside of living plants.
- (5) In artificial inoculations these three bacilli multiply not only at the point of inoculation but spread into the neighboring parts.
- (6) Although these three bacilli spread from the point of inoculation to adjacent parts of the tissue, they do not extend widely, that is, the whole organ or the whole plant is not infected by artificial inoculation.
- (7) The part of the leaf injured by the microbes sometimes may be identified macroscopically. The injured spot in the leaf differs from the healthy part by being lighter green. Sometimes on the part injured by *Bacillus prodigiosus* brick-red spots or stripes are noticed along the track of the injury. [Probably a host-reaction.]
- (8) The disease-creating microbes spread in the plant by way of the intercellular passages, and the size of the microbe is of great importance in this regard. The smaller it is, the easier it spreads in the tissue. For this reason *B. prodigiosus* spreads farther than the others.
- (9) The author could not observe that motility in any way favored its spread.
- (10) The walls of the cells do not absolutely prevent the entrance of the microbes into the cell.
- (11) The protoplasm of the cell may afford a medium for the growth of the microbes.
- (12) The dead and dry tissue does not afford a good medium but the dead and juicy cells afford a very convenient soil for their development. The microbes were also alive in the living cells but preferred the dead ones.
- (13) The bacillus of the Siberian plague multiplies vigorously during the first few days in the leaves of *Agapanthus* and grows out into a thread. At the end of the first week there is an inclination to form spores, which in course of time becomes more distinct. Many spore chains were visible on the eighteenth day, together with separate spores and nonspore-bearing threads. Filaments and spores occur not only at the point of inoculation but also between the healthy cells of the spongy tissue of the leaf, and in the cells themselves. Some of these threads stain well with gentian violet, others do not. Those which do not stain with gentian violet, stain afterward with carmine (double stain) and are refractive. Still others stain only in parts, or are not stained, and look like bright, pale, drawn threads. Slides made 42 days after inoculation still showed numerous vegetative forms of the plague bacillus, together with spores and spore-bearing threads.

(14) In *Agapanthus* leaves 26 days after inoculation the bacilli and filaments of the Siberian plague were found showing great changes. On the unstained slides their color was somewhat yellowish and they were noticed on account of their refraction which was like glass or galena. These threads were two or three times the diameter of normal filaments and they were constricted, *i. e.*, they had lost their normal filamentous form.

(15) Sowings of infected parts of the leaves of agapanth on nutrient gelatin or boiled potatoes on the sixteenth and forty-second day after inoculation gave a typical culture of the Siberian plague bacillus.

(16) The inoculation into mice of portions of similar leaves 16 and 42 days after the inoculation of the plant caused the death of the animals from the Siberian plague.

(17) The typhoid fever bacillus multiplied in the leaves of wheat and agapanth only during the course of the first days after inoculation, gradually dying out. This dying out was shown (a) by its not taking Loeffler's or Ziehl's stain, (b) by the presence of involution forms, (c) by its failure to grow in cultures.

(18) Of all the microbes investigated *B. prodigiosus* multiplied most energetically and after the manner of the Siberian plague, that is, in the intercellular passages and in the living cells adjacent to the point of inoculation.

(19) The dying out of *B. prodigiosus* was not noticed even at the expiration of 32 days from inoculation. On this date transfers from inoculated parts of the leaf into nutrient gelatin and boiled potatoes gave a typical culture.

(20) Plants in the course of their growth may mechanically throw out the microbes from shallow layers to the surface.

(21) When wheat is grown on soil infected by disease-creating microbes many of the microbes may enter into the root-system, the smaller ones getting in the easier.

(22) When wheat is grown on soil infected by a mixture of microbes all of them may be found in the tissues of the root.

(23) The passage of the microbes from the infected roots of wheat into the stems and leaves was not observed.

Russell (p. 6) states that he could not confirm Lominsky's results with *Bacillus prodigiosus*, *to wit*, the production of red spots and stripes in the injected plants, but inasmuch as he did not experiment with the same plants as Lominsky, his experiments can not be considered as a refutation of Lominsky's statements.

Russell also states that he failed to verify some of the results obtained by Lominsky with animal parasites injected into plants, but here again his experiments are not strictly comparable since he used different plants.

He also obtained different results from watering the soil with "dilute infusions of the different germs." In this case Russell does not state what plants he used, but presumably not wheat, from a remark on the following page—"This result would have been much more convincing had he [Lominsky] used larger plants than wheat."

Russell found *Bacterium pyocyaneum* present in large numbers at the point of inoculation in begonias after 69 days; in geranium after 32 days, and in *Penthorum* after 36 days. The anthrax organism was absent from geranium (*Pelargonium*) at the point of inoculation after 38 days, and was only sparingly present in lima bean after 11 days and in *Echinocactus* after 5 days. *Staphylococcus epidermidis albus* was not recovered from the point of inoculation in geranium after 40 days. *Staphylococcus pyogenes aureus* was also dead in geranium after 42 days, but was recovered very sparingly from lima bean after 13 days. *B. cholerae gallinarum* was moderately abundant in geranium after 18 days. The organism of *Schweine-suche* was present in large numbers in geranium after 17 days. The diphtheria organism was not found in geranium at the point of inoculation after 10 days. He has the following paragraph on the result of his experiments with *Bacillus amylovorus* and *Bacterium avenae*.

"The pear-blight germ grown in a begonia-plant for 30 days showed at end of that time large numbers at inoculation point, but not distributed throughout the plant. The same result was found when injected into *Phaseolus vulgaris* for 30 days, also in *Ph. lunatus* for 16 days. In *Tradescantia alba*, no trace could be found at the end of 60 days' incubation in this tissue. *Bact. avenae* was injected into tissue of begonia, onion, corn, wheat, and squash, but in no case was any pathological change macroscopically observable. The bacilli were not killed out in the plant-tissue, however, as they were isolated from begonia and squash in large numbers, after 30 days' incubation in these tissues, but their presence was confined to the tissue contiguous to point of introduction."

Concerning the general conclusions to be drawn from his own observations, Russell has the following:

"The results of the foregoing inoculation experiments made with various forms of micro-organisms, saprophytes as well as parasites (both for animals and vegetables), show that these germs in many cases are able to live in the plant-tissues for a considerable length of time. A number of the different forms, particularly saprophytes, are able to grow and spread throughout the plant to a limited extent. Of the parasitic species tested, very few showed any tendency to thus spread. Even

those forms that are natural parasites of certain higher vegetable species showed no power to spread in plants which were not their natural hosts, but they were able to live at inoculation-point for a considerable time. \* \* \*

"The distribution of the micro-organisms in the plant-axis, as determined by culture experiments, always took place in an ascending direction. This distance varied from 30 to 50 mm. from point of introduction, but in no case were bacteria found more than 2 to 3 mm. below inoculation-point."

Charrin (1893) injected *Bact. pyocyaneum* into *Pachyphyton bracteosum*, a plant of the family Crassulaceae. The inoculations were made into the fleshy leaves. This organism lived for some time and multiplied (mostly in the intercellular spaces). The leaves of the plant, after the germs had grown in them 15 to 30 days, became wrinkled, lost color, and fell off; the acidity of the leaves lessened in proportion as the bacteria multiplied. Even when large numbers of the organism were introduced (0.25 to 0.5 cc. of a liquid culture) it remained alive a short time only (2 to 3 weeks, more or less) and when only 1 to 2 drops were inoculated 8 to 12 days usually sufficed to kill the organism, especially if a weakened germ was used.

An interesting paper, especially the last half dozen paragraphs on immunity.

Kasperek and Kornauth (1896) experimented with *Aplanobacter anthracis* on oats, barley, wheat, rape and maize. Their method of procedure was as follows:

Sterilized flower-pots were filled half full of sterile soil and each watered with 10 cc. of bouillon containing large numbers of spores and threads of the anthrax organism. Sterile seeds of the above mentioned plants were then embedded in this moistened earth and the pot filled nearly full with additional sterile soil. The surface of the seeds was sterilized by soaking for a short time in 2 per cent mercuric chloride solution which was removed by washing in sterile water, alcohol and ether. Then they were put for 24 hours into nutrient bouillon at blood temperature to be sure that their surface was actually sterile, and the seeds in those tubes which clouded were rejected. The germinating power of seeds thus treated was not injured. After two months in the first series, and after three months in the second series the experiment was broken off and the earth and plants examined microscopically and bacteriologically. A microscopic examination of the soil showed that anthrax spores were abundant not only in the part which was watered, but also in the upper layers of the soil even to the surface, but no anthrax threads or rods could be found. Samples of this soil produced typical anthrax when inoculated into white mice and also gave numerous anthrax colonies when sowed in plate cultures. The unwashed underground parts when inoculated into mice also produced typical anthrax, but when these roots and stems were first washed with mercuric chloride, alcohol and ether, the same as the seeds had been, they did not produce any disease in mice when inoculated, and the agar plates also remained sterile. Moreover, a microscopic examination of sections made through the roots and other parts of these plants grown in anthrax infected earth likewise showed complete absence of the anthrax organism in the tissues of the plants.

Kornauth (1896) continued and extended these experiments, including *Streptococcus pyogenes* with anthrax in order to have a very small Schizomycete for comparison with the large one.

The results were the same. Kornauth used for his experiments seeds of maize and peas. These were washed in 2 per cent mercuric chloride water, alcohol, and ether, then put into sterile bouillon in Petri dishes and incubated for 2 days at 37° C. If the bouillon remained clear then it was inoculated with either *Aplanobacter anthracis* or the streptococcus. The cultures succeeded admirably and appeared on microscopic examination to be pure. After about 3 weeks when the seedlings had reached the length of about 2 cm. the experiment was broken off. After washing the seedlings in mercuric chloride water, alcohol and ether to render the surface sterile, they were crushed with antiseptic cautions, and inoculated into mice (those used with anthrax) and also put into sterile bouillon. Sections of these seedlings were also prepared for microscopic examination. The result of the experiment was that the mice did not contract the disease, the bouillon remained clear, and the sections showed no trace of any bacteria.

His conclusion, therefore, is that the plant under normal conditions is a perfect filter for bacteria, that only a few species of bacteria can penetrate the uninjured plant tissue, and these only under very special conditions. He next undertook to determine whether the organisms would multiply in injured tissue, i.e., in wounds, as stated by Lominsky. For this purpose he selected the following

bacteria: *Micrococcus cinnabarcus*, *Aplanobacter pneumoniae* (Weichselbaum), *Streptococcus pyogenes*, *Bacillus coli*, *Bacillus prodigiosus*, *Mycobacterium diphtheriae*, *Bacillus typhosus*, *Aplanobacter anthracis* (spores and filaments) and *Actinomyces*.

For each one he used two specimens of onions and hyacinths well provided with leaves, and three sorts of cactus. The places where the wounds were to be made were first painted with mercuric chloride, alcohol and ether to destroy the surface organisms, then with a pair of sterile shears the wound was made and through the opening by means of a platinum loop the culture was inserted. The wound was immediately closed, the exuding excess of culture removed with a sterile knife and the wound fastened together with collodion. As a rule the wound healed well.

After 8 days the infected spots were sampled with a flamed corkborer, their infectiousness tested on animals, and the presence of the organism determined both by cultures and by examination of sections. With the next larger corkborer, a cylinder-mantle was also removed and transferred to bouillon. In the cylinders that were used for inoculations and sections the following organisms were found living: *Aplanobacter anthracis*, *Bacillus prodigiosus*, *B. coli*, and *M. cinnabarcus*. The following were dead: *Streptococcus pyogenes*, *Mycobacterium diphtheriae*, *Bacillus typhosus* and *Aplanobacter pneumoniae*. *Actinomyces* transferred to agar also failed to grow. The cylinder-mantle, which was about 5 mm. thick, left the bouillon clear. Anthrax was found only in spore form in the plant tissues. These wounds were all superficial. The author then tried whether inoculations into deeper wounds would have any different results. For this purpose he used a needle with which he made deep punctures introducing into them the organism. The general method of procedure was the same, sterilizing the surface and finally covering the wound with collodion. Tests were then made after various periods. Again the anthrax organism was found to have sporulated and the spores were fully infectious at the end of 4 months. The diphtheria organism and the pneumonia germ were noninfectious at the end of 48 hours. The typhoid bacillus at the end of 5 days was non-infectious, and *Bacillus coli* at the end of 8 days. *Micrococcus cinnabarcus* and *Bacillus prodigiosus* dried out but remained alive a long time. *Streptococcus pyogenes* also remained alive, but showed on the ordinary culture media only a slight or weakened growth. The sections showed that the diphtheria organism had taken on involution forms, while the *Actinomyces* had fallen into a granular detritus. These experiments were made in warm and dry air.

This author also tried some experiments in moist air, using for this purpose *Aplanobacter anthracis* (spores and threads), and *Micrococcus cinnabarcus*. The inoculations were into buds and the plants were kept in a moist chamber. After some days fungi appeared on the inoculated places and these seem to have quickly killed the bacteria, as the latter could not be recovered in poured-plates.

Kornauth's conclusions therefore, are just opposed to Lominsky's: "The bacteria introduced into the living plant under favorable conditions as to warmth, exclusion of foreign organisms, etc., have never shown any multiplication and just as little any staining of the inoculated spot (through chromogenic bacteria) or a loss of color of the surrounding tissues."

Zinsser's paper appeared in 1897. It deals mostly with the root-nodule organisms of the Leguminosae, but there are also detailed experiments with other bacteria. The work was done in Pfeffer's laboratory.

Zinsser gives a very interesting table showing the behavior of various bacteria when introduced into plant tissues. In one instance *Bacillus prodigiosus* yielded cultures after remaining in a bean stem 96 days. In general, *Bacillus subtilis*, *B. megaterium*, and *B. prodigiosus* were most resistant, being frequently found alive in the stems and leaves of various plants after 14 to 48 days. Other organisms were destroyed more speedily. Zinsser also inoculated animal pathogenic forms into plants. He experimented mostly with *Aplanobacter anthracis*. This lived in various plants such as beans, *Cyclamen*, *Abutilon*, *Sempervivum*, and *Barbarea* from 14 to 28 days. In several cases it was dead after 27 to 28 days. Even the more resistant species did not multiply extensively or behave like parasites. All lost their ability to grow after a longer or shorter period and perished. Concerning their spread in the tissues the author says:

"Now and then according to the microscopic appearance the injected bacteria appeared to have multiplied for a short time, and they were able also to penetrate into the neighboring tissue, but this power of translocation is not great, for even a few centimeters away from the point of inoculation I could not afterwards demonstrate bacteria."

The following notes are from Hartleb's paper:

Hartleb, who worked in Stutzer's laboratory in Bonn, states (1898) that he experimented with the bacteria of the foot and mouth disease, using their organism, and Siegel's organism. The inoculations were made into the stems of beans, *Vicia faba*, potatoes, and peas. He also inoculated pods

of beans and peas. His method was to wash the surface with mercuric chloride water, then with sterile water, after which a longitudinal incision was made with a sterile knife, the wound pried apart a little and the platinum needle carrying the infectious material introduced. Unlike Kornauth and Kasperek he did not cover the wound with collodion but left it exposed to the air so as to have the conditions as nearly as possible like natural conditions. He states that the organism remained alive in wounds for a long time and multiplied. When the seeds were wounded most of them passed over into a slimy rot which contained for a long time extremely well developed living bacteria of the form introduced. Bacteria from these wounds produced the typical disease when inoculated back again into animals (white mice and finally guinea pigs). In the dried parts both of stems and pods, the organism in its resting form was found to be alive and infectious to animals at the end of 6 months. "The inoculation cuts had for the most part a rusty brown appearance [host reaction], but not rarely in some cases there was also a slight accumulation of the slimy substance to be observed which was found to be an aggregation of bacteria into a slimy mass."

His conclusions are as follows:

- (1) Our bacterium cultivated upon acid nutrient media can develop further in living plant parts.
- (2) It is capable of remaining alive not simply for a short time, but even upon dead plant parts with the help of its resting forms, perhaps to maintain a prolonged capability of growth, without actually penetrating directly into the cell-tissue and multiplying inside of the same.
- (3) That, in consequence of this preponderating parasitic manner of life, it is able, when carried back to animals to cause the infection and death of the same.

It is stated that the organism was also cultivated on carrots, both sterilized and unsterilized, where it formed at first a slime, and afterwards a dry layer, and in one instance a guinea pig was infected by feeding upon such carrots. The inoculated parts of the plants either healed over with more or less callose, or else there was a direct death of the cell-tissue in the vicinity of the wound. Generally the organism appeared in unmixed growths in the wounds, sometimes in vegetative forms as rods, and sometimes in the resting form. Only scattering wild yeast-cells accompanied it.

Hartleb states that the cultures he used had lost most of their power to destroy animals and consequently this passage through plants increased their virulence. Just what he worked with is not apparent, as the cause of the foot and mouth disease is still believed by most pathologists to be undetermined and probably ultra microscopic (Vide Kolle und Wassermann, Jena, 1904, Bd. IV, 2ter Teil, p. 1325).

Laurent (1899) maintained that he was able by special treatments to cause *Bacillus coli* to become a plant parasite, but this claim is still disputed. In this connection see page 42.

John R. Johnston working in the writer's laboratory on the coconut bud-rot of the West Indies has obtained evidence that it is due to an organism indistinguishable from what ordinarily passes for *Bacillus coli* (Phytopathology, Vol. I, No. 3).

Ellrodt's paper (1902) relates to the possible transmission of human and animal parasites by means of plants. He states that *Bacterium pyocyaneum* can enter plants through wounded roots, but not through sound ones. This conclusion rests on the following experiments.

In a series of flower pots, oats, beans, vetches and peas were planted. When these were about 20 cm. high, the earth was watered with a suspension of *Bacterium pyocyaneum*. The same experiment was undertaken with *Viola odorata*, *Paconia officinalis*, and *Iris sibirica*. Cultures were made the next day by cutting out a piece of the plant with sterile knives, thrusting a platinum needle into the exposed tissues and streaking the sap on agar and glycerin agar. As the cultures gave absolutely negative results, the earth was again wet with a bacterial suspension of the organism, and after 4 or 5 days streaks were again attempted on agar in the same manner. These likewise remained sterile. Samples of the soil, on the contrary, yielded numerous colonies of *Bacterium pyocyaneum*.

Erlenmeyer flasks containing a culture fluid of the following composition: distilled water 1000; asparagin 5; sodium acetate 5; potassium phosphate 2; sodium chloride 2; magnesium sulphate 0.1, were now inoculated with *Bact. pyocyaneum*, and incubated for 5 days, during which time there was luxuriant growth and appearance of the characteristic blue-green color. Bean plants taken from pots were now introduced into this fluid. After some days leaves were cut off with a sterile knife, a platinum needle was thrust into the leaf-stalk, and streaks were made on glycerin agar. These remained sterile. Some days later the cultures were repeated, being taken this time from the interior of the stem at 15 cm. from the roots. One plant yielded a pure culture of *Bact. pyocyaneum*. Those

plants which yielded no bacteria at this level were now cut off just above the roots and a third set of tubes inoculated from the inner tissues, all of which now yielded this organism. As this positive result was not in accord with the previous experiments and might perhaps be attributed to injury of the roots during removal from the pots, the following experiment was undertaken.

Beans were planted in a nutrient fluid consisting of: water 1000; potassium nitrate 0.5, potassium phosphate 0.2, magnesium sulphate 0.2, ferrum sulphate 0.1. They grew well in this fluid and when about 20 cm. high a culture of *Bact. pyocyaneum* was added. The roots of some of the plants were purposely injured, while the others remained sound. After some days cultures were made from the interior of the plants. These showed the presence of *Bact. pyocyaneum* in all the injured plants, and in none of the uninjured ones.

Ellrodt's conclusion, therefore, is that bacteria can not penetrate sound roots, but may enter through broken ones, and that since root-injuries are common occurrences in soil, it is not yet certain that pathogenic bacteria can not enter the plant from infected soils.

Clauditz criticises Ellrodt for not telling in what tissues the bacteria occurred. Furthermore, inasmuch as he does not state that he washed or otherwise removed the bacteria from the surface of the plants, he may really have got his results from surface organisms, which were dragged into the tissues. Certainly, the surface of his plants, particularly the parts near the roots and consequently near the bacterial fluid, should have been flamed or otherwise disinfected. In the last mentioned experiment, however, he probably did not get his results from surface organisms because his checks were sterile.

Clauditz (1904) made a series of experiments with the typhoid bacillus to learn whether infection through plants is possible and especially whether this organism can penetrate into the interior of plants. In certain respects his statements also are vague.

Clauditz used the plants which are commonly eaten raw, *viz.*, radish, cress, and lettuce. The soil was taken from the yard of the Hyg. Institute of the Royal University of Berlin. To imitate as nearly as possible the conditions of the soil in the sewage fields several glass tubes were thrust into the earth a depth of 8 cm., and 24-hour old bouillon cultures of the typhoid organism were poured into these tubes every other day. After 8 days, repeated attempts were made to recover the organism from the soil, but these failed in spite of renewed infections with a fresher isolation. These cultures were made both from the surface and from 4 to 6 cm. down.

He states that it is difficult to isolate the typhoid organism from the earth because in most cases this organism quickly perishes when brought into competition with the bacteria of the soil. Following Rullmann's advice he mixed the infected soil with double its quantity of sterile bouillon and incubated at 37°, but always the soil organisms got the advantage and the typhoid bacillus was not to be recovered in this way. He then tried to accustom the typhoid organism to the soil bacteria in bouillon cultures by adding to sterile bouillon a loop of a 24-hour bouillon culture of *B. typhosus* and 2 loops of soil and exposing for 24 hours at 37° C. From this tube 3 loops were then transferred to a second tube which was incubated for the same time and at same temperature, and so on for 10 tubes. After 5 days the typhoid organism was not demonstrable in the first tube, and not after 24 hours in the second, while in all the others the results were negative.

The strain isolated from the second tube was designated "Typhus Erde I." With this a second series of 10 tubes was inoculated in the same way as before. The results from this set of tubes were all positive, and even after a half year the typhoid organism was easily demonstrated in tube 10. Along with it were present a variety of other bacteria, *Bacillus subtilis*, *Bact. fluorescens liquefaciens*, cocci, etc.

A second set of soil inoculations was undertaken with this strain, "Typhus Erde II," the bouillon cultures being now poured into the soil after dilution with sterile distilled water. It was now easy to demonstrate the bacillus in the soil and a strain so isolated was called "Typhus Erde III." The latter was now used for all the subsequent experiments.

After the organism had been isolated from the soil and when the plants were 5 to 8 cm. high, they were cut off close to the earth with a sterile knife, washed one-half hour in sterile water, bruised in a sterile mortar with sterile bouillon and then incubated for 24 hours at 37° C. Streaks were then made on Drigalski-medium, one out of four being positive. The experiment was repeated with the precaution first to put the plants in a 1:100 solution of mercuric chloride (time not stated) and then wash them thoroughly in sterile water. All the tests were now negative. To avoid the objection that the mercuric chloride may have penetrated the plants and killed the bacteria, the experiment was repeated, the surface of the plants being sterilized this time (so far as regarded *B. typhosus*) by



dipping them for 10 to 20 seconds in hot water (90° to 70° C.). All the results were again negative. Some soil bacteria appeared in all the plates which ever way treated. The above mentioned plants had uninjured roots. In additional experiments the roots of the plants were now injured, but the results of the cultures [time intervening not stated] were still negative.

New experiments were planned in which the bacterial cultures were let into the subsoil by rubber tubes having side openings. The soil and drains were in large flat pans; on these, earth was laid and this was sowed with lettuce, radish, and cress. When the plants were about 4 cm. high, the infection of the soil through the drains was begun, the fluid being uniformly distributed in all parts of the dishes. The roots of some plants were injured, others not. The results of the subsequent cultures were negative in both cases. It is not stated whether the pans (Schalen) were *zinc* or *copper*.

An experiment was now made with peas. The conditions of the experiment were as before, except that the soil was infected with the typhoid bacillus before the peas had sprouted. The roots were injured when the plants were 4 cm. high. When they were 10 cm. high the plants were cut off close to the earth, washed in sterile water, crushed in a mortar and streaks made at once on Drigalski-media or Endo-media. The results were as follows: 1 (direct streak)—positive; 2 (Ficker-Hoffmann's method)—positive; 3, 4 (direct streaks)—negative.

Five stems were now examined by culture in their upper and lower parts. Three gave negative results, two showed the bacteria in the lower end (pieces 3 cm. long), but not above. The bouillon in which the stems were washed yielded the bacillus when the interior of the stems did not.

There can at least be no doubt, therefore, that bacteria were brought up out of the soil on the surface of the plants, since the plants were watered with the bacillus entirely from below.

Another experiment was now made, the soil being first wet from below after the plants were 10 cm. high. Some days later the roots were broken, and a few days afterwards [scant time allowed] the plants were examined in the same way as before. Only negative results were obtained. The conclusion reached is that the typhoid organism occurs on the outside of the plant and sticks so fast that it can not be washed away.

Radish and pea plants were now wet with a suspension of the same culture of the typhoid bacillus for comparison. After 14 days the bacillus was found abundant on the leaves and stems of the peas in spite of direct sunlight, but had disappeared from the radish by the fourth day. The radish leaves appeared to be an unfavorable surface. The surfaces of radish roots grown in infected soil were now tested for the presence of the typhoid bacillus after they had been washed until all visible dirt was removed. In all cases the typhoid bacillus was found in abundance on the surface of such roots.

#### PLANT PARASITES INOCULATED INTO ANIMALS.

In 1895 Ostrowsky reported as moderately pathogenic to rabbits a short rod-shaped Schizomycete said to have been isolated from the browned interior of grape-stems.

The rabbits were inoculated intravenously (quantity not stated) a slight fever supervened, there was rapid loss of weight (400 grams in 8 to 10 days) and on autopsy there were sometimes small miliary abscesses in the spleen or liver.

The organism is not well described. It liquefies gelatin; the colonies are moist, soft and whitish on agar. It is apparently aerobic. In old gelatin cultures there is a brown stain. No evidence is offered in support of the statement that it is parasitic on the vine, and as a matter of fact Viala and Ravaz state that it was not, *i. e.*, no disease of the vine could be induced with it by means of inoculations.

In 1899 Charrin and Viala state that when the microbe of gélevure, otherwise known as Mal nero, Commose bacillaire, Maromba, Maladie d'oléran, etc., was first inoculated it caused at most the death of some fish, but by repeated inoculations into rabbits it caused frequently a slight enteritis with some fever and loss of appetite, etc., ending in recovery in the greater number of cases. The germ is said to require education. It is not described. Owing to its speculative character and the absence of all details as to the exact nature of the experiments, the paper does not tend to win the confidence of the reader. There are many opportunities for error. Moreover, the etiology of Mal nero itself is still in doubt, with the probabilities against its being of bacterial origin.

According to Dr. V. A. Moore *Bacillus cloacae*, supposed to be the cause of a disease in maize, has no effect on experimental animals except when injected into the blood stream in large quantities.

Harding inoculated *Bact. campestre* into animals with negative results.

Harrison inoculated *Bacillus solanigrans* into guinea-pigs and rabbits without positive results (see Vol. III, Basal Stem-rot of Potato).

The following experiments with fishes in water containing bacteria of plant diseases were undertaken for me in 1905 by M. C. Marsh, bacteriologist of the Bureau of Fisheries, in Washington. I prepared the cultures myself. The summary is his:

*Bacillus aroideae*.—About 25 liters Potomac tap water in glass aquarium jar, with constant flow of air in small bubbles delivering at the bottom. Two sunfish, 1 small goldfish, and 1 mummichog were used with 14 cc. of a well-clouded 2-day bouillon culture of *B. aroideae* introduced on first day, February 17, 1905; March 5, 15 cc. of a 5-day culture added. March 13, after 26 days, all fishes alive and in good condition.

March 5, injected largest of the above sunfish behind eye with about 0.5 cc. of a 5-day well-clouded bouillon culture of *B. aroideae*; producing great exophthalmia. Sunfish remained in jar containing *B. aroideae* mixed with water. March 13, fish alive, eye normal; time 8 days.

*Bacteria of carnation leaf spot*, February 19, about 25 liters Potomac tap water in glass aquarium, aeration as above. One small black bass (5 inches), 2 sunfish, 1 mummichog. February 20, added two 20-day bouillon cultures of carnation bacteria, one 5-day bouillon culture, and one 20-day agar slant culture; February 28, added three 9-day bouillon cultures.

March 13, after 21 days, all fish in good condition.

Temperature of water 14.5° to 22° C. No change of water during experiments. Fishes fed very sparingly.

In transmitting the above report Mr. Marsh made the following comment:

I send you herewith a statement of the effect of the plant bacteria on fishes, from which it appears that the effect is *nil*. I did not make a direct inoculation with the carnation rot, on account of the result with the presumably more dangerous calla rot. It is not likely that these organisms would harm any fishes, though I was unable to try trout. The eye inoculation should have taken if there was any pathogenicity about the calla rot for fishes.

#### INOCULATIONS OF *BACT. TUMEFACIENS* INTO FISH AND FROGS.

In the spring of 1908, the writer made fourteen sets of inoculation experiments on fish and frogs with pure cultures of *Bact. tumefaciens* derived from tumors on the hothouse daisy (*Chrysanthemum*) to determine whether this organism would induce similar abnormal growths in cold-blooded animals, experiments on warm-blooded animals being considered unnecessary because of the low maximum temperature of the organism (about 36.5°C.).

These inoculations were carried on in Washington in houses belonging to the Bureau of Fisheries with trout and roach kindly placed at my disposal from the stock tanks and with frogs bought from a Washington dealer. With exception of those used in Experiment IX, the trout were 8 to 10 inches long and were ordinary brook trout (*Salvelinus fontinalis*). The roach (*Abramis chrysolaemus*) were about 6 inches long. There were no checks on the roaches or frogs. The checks on the trout consisted of a school of about 100 fish of the same age and condition, and of which those I took had previously formed a part. These were in one of the ordinary exhibition tanks of the Bureau along with some rainbow trout. They were not checks in the strictest sense of the word because they were not wounded in any way.

*Inoculations of March 20, 1908*.—These were made from four agar streak cultures 48 hours old. None of them were hypodermic injections.

I. Two trout. Each two needle-pricks in the eye-socket.

II. Two trout. Each three needle-pricks in the region of the anus.

III. Two trout. Each three or four needle-pricks in the fleshy fin (adipose).

- IV. Two trout. Each two shallow pricks in the throat outside near the junction of the gill arches.  
V. Two trout. Each inoculated in the eye-socket. This time the skin was cut with a scalpel and a 2-mm. loop of the white bacterial slime was inserted.  
VI. Two trout. Each inoculated inside of the mouth at the base of the tongue by means of several needle-pricks.  
VII. Four roach. Each inoculated in the eye-socket. The skin was cut and a 2-mm. loop of the bacteria inserted.  
VIII. Two roach. Each received several needle-pricks in the vicinity of the anus.  
IX. Fifty salmon trout fry (2 to 3 cm. long). They were put over night into 2 liters of water into which the remnant of the 4 agar cultures had been washed. The next morning they were transferred to running water in the ordinary shallow hatchery boxes.

*Inoculations of April 4, 1908.*—For these, 5 slant agar cultures 3 days old were used. The copious growth was washed off into 20 cc. of distilled water, making a milky suspension. All of the inoculations were made with the hypodermic syringe.

X. Three leopard frogs. Each received  $\frac{1}{2}$  cc. of the very cloudy fluid. This was injected into the muscles of the right thigh.

XI. Three leopard frogs. Each received  $\frac{1}{2}$  cc. under the skin on the abdomen. In one of these it was thought that the needle entered the abdominal cavity and for this reason it was kept separate, but the result was not different.

XII. Two leopard frogs and 3 green frogs (*Rana clamitans*). Each received  $\frac{1}{2}$  cc. in the right eye-socket.

XIII. Three brook trout. Each received  $\frac{1}{2}$  cc. in the eye-socket.

XIV. Three brook trout. Each received  $\frac{1}{2}$  cc. of the very cloudy bacterial suspension. This was injected into the peritoneal cavity, the needle being set in just behind the ventral fins.

XV. The virulence of the cultures was determined by making inoculations on four young daisy plants. These promptly contracted the disease. On June 1 these plants bore, where inoculated, tumors which were over an inch long by 0.50 to 0.75 inches broad and thick.

*Results:* The results so far as tumor production is concerned were either negative or uncertain; all the fish have not been sectioned. The experiment was complicated by the discovery after the inoculations were begun that some of the fish were suffering from carcinoma of the tongue, gills and thyroid region. Thenceforth, I examined each fish and inoculated only such as appeared to be sound, but nevertheless some of them may have then been about ready to develop such tumors as subsequently appeared. On March 25, 1908, I caught and examined 50 of the 100 check fish and found 3 with carcinomatous throat tumors. Additional cases of this disease appeared in the checks, especially as the season advanced. Consequently the tumors which developed on the inoculated fish may have been due altogether to this disease, at least under the circumstances I could not be certain that they were not so caused.

The frogs proved very resistant. None developed tumors. Most were finally chloroformed at the end of the experiment. The few that died earlier gave no plain evidence of being in any way injured by the bacteria.

Of the roaches one died the day after inoculation. The rest died in from 17 to 32 days. None of the latter developed tumors. All were more or less inflamed, both roaches and trout.

The inoculated trout (except the fry which showed no signs of disease attributable to the bacteria) died off faster than the checks in the main tank. They were, however, not under altogether the best conditions, *i.e.*, they were rather too crowded at the beginning of the experiment and the water was several degrees too warm toward the close of the experiment, but at the same temperature as that given to the check fish. If I were to do over this experiment in a climate like that of Washington, I would begin in the autumn, so as to allow the experiment to run for at least six months in cool weather.

Of the inoculated trout one died at the end of 15 days. The rest (with one exception) died in from 30 to 40 days from the time of inoculation. The relative rate of death of inoculated and check trout is shown in the following table:

TABLE SHOWING DATE OF DEATH AND SYMPTOMS OBSERVED IN TROUT.

Inoculated Trout.		Check Trout.	
Date.	No.	Remarks.	Remarks.
1908			
Apr. 4	VI, 1	Congestion in region of anus, base of tongue, liver, heart.	From Mar. 20 to Apr. 29, no deaths among the 100 check fish.
Apr. 22	II, 1	Eye-socket inflamed. Liver diseased.	During this period 10 deaths among the 18 inoculated fish.
Apr. 23	VI, 1	Hard yellowish rough tumor on tongue (the only hard tumor). Eye-socket inflamed.	On Mar. 25, 3 showing throat tumors were separated from the rest of the checks.
Apr. 25	XIV, 1	Walls of lower intestine inflamed, ulcers on inner belly wall and externally at root of pectoral fin and on outside below anal fin.	
		Throat sound.	
	II, 1	Anemic. Ulcerous tongue and inner belly wall.	
Apr. 28	IV, 1	Anemic. Eye-sockets inflamed. Vicinity of thyroid inflamed and slightly swollen.	
	V, 1	Eye-socket inflamed. Liver white-mottled. Spots on gills and region of the thyroid inflamed.	
Apr. 29	IV, 1	Stomach and intestines much inflamed. White patch on liver. Back part of throat inflamed, i. e., below the tongue. Inflammation in throat where needle-pricks ended; None outside.	
	XIV, 1	Marked inflammation of abdominal wall where needle entered. Needle wound healed externally. Inflamed spot at base of tongue. Lower intestine much inflamed.	
Apr. 30	IV, 1	Bloody patches on lower intestine. Ulcerontail.	Apr. 30 1. The first check to die. Tumor in throat.
May 1	III, 1	Several small ulcers on surface. Throat sound, liver diseased, inflamed patches on inner wall of abdomen.	
May 2	I, 1	Small abrasion on surface, also a Saprolegnia patch (3 sq. in.). Gills, stomach and intestine congested. Eye-socket badly inflamed.	
May 4	XIII, 1	Eye on inoculated side is white-clouded, swollen inflamed tissue at base of eyes, especially on inoculated side. Marked congestion of the inner wall of abdomen in lower part; tongue somewhat swollen and red.	
May 5	V, 1	Small sore spot at roots of the tail. Throat sound. Membrane covering the intestine and eye-socket highly inflamed. Also inflamed places on the inner lower belly wall.	
May 7	III, 1	No tumor on the adipose (where inoculated). Base of tongue swollen and inflamed outside and inside.	May 6. Two, with cancerous growths in throat.
May 11	XIII, 1	Throat and gills sound, eye-socket inflamed, viscera swollen and inflamed. Inner belly wall badly inflamed.	May 8. 1. Cancerous thyroid May 9. 1. Mouth sore. May 11. 7. Two have sores in the mouth May 14. 3. One anemic and with an ulcer on the gills. Weather hot for last three days. May 15. 1. Cancer in thyroid region May 16. 4. One has a cancerous throat May 19. 7. Not dissected.
May 19	XIV	One alive on this date when experiment was abandoned.	

Since the above paragraphs were written the most hopeful portions of the inoculated trout have been infiltrated, sectioned, stained, and studied, with the following results (the figures in parenthesis refer to the preceding table).

519 (April 28, V). Ulcer on inner wall of abdomen. Proliferations too regular for sarcoma. Probably not malignant.

520 (April 25, XIV). Hypodermic. Ulcer on inner belly wall near entrance of needle. Sarcoma (?). Very suspicious.

546 (April 23, IV). Proliferations—not malignant.

549 (April 25, II). Tumor on tongue: Adenocarcinoma.

621 a (April 23, I). Portion of tongue. Probably not malignant. Some of the cartilage has an abnormally large number of cells in it (chondroma?).

622 (April 12, II). Inflamed part of inner wall of abdomen. Proliferations but nothing definite.

642 (April 19, XIV). Sections of small swellings on inner belly wall where inoculated. May be accounted for as simple inflammation. Later: There are giant cells in it.

625 a (May 4, XIII). Hypodermic, eye-socket. Ulcer at base of eye. Typical giant cells, but possibly foreign body giant cells.

#### DO PLANTS HARBOR ANIMAL PARASITES?

In a paper published in 1889 in the *Comptes Rendus* of the French Academy, Dr. Domingos Freire undertakes to show that roses and various other common flowers harbor bacteria, some of which are pathogenic to man. All this paper really proves is that which

was already well known, *viz.*, that bacteria are commonly present in the air and consequently liable to be found on the surface of everything, not excepting the surface of the flowers with which the author experimented. Florists and flower lovers need give themselves no particular uneasiness, since Dr. Freire's conclusions do not necessarily follow from his experiments. As to the correctness of his identification of species, the paper offers no means for judging. The author's conclusions are:

(1) That animal pathogenic bacteria, *Streptococcus pyogenes*, *Bacillus pyocyaneus*, etc., are normally present in flowers which can, as he says, "noteworthy store up numerous germs, which may subsequently finish their development in the better adapted tissues of animals or plants;" (2) that there is some hidden relation between the colors of flowers and the bacteria found on them, *e.g.*, the color of the colonies of *Leptothrix ochracea* and the very pale rose color of the Rothschild rose, or the egg yellow color of *Micrococcus cruciformis* and the yellow of *Hibiscus rosa-sinensis*; (3) that certain bacteria, called by him *osmogènes* "reproduce odors analogous to those liberated by the essential oils of the flowers where they live."

Two new species are very imperfectly described, *Micrococcus cruciformis* from the anthers of *Hibiscus* and *Bacillus gallicus* from *Rosa gallica* (*centifolia*). From *Ipomoea quamoclit* L. he isolated an organism having the characters of *Micrococcus salivarius pyogenes* and another identified as *Spirillum plicatiale*. From the flower of the peach he also obtained something identified as *Bacillus pyocyaneus*.

Uffelmann's experiments (1892) showed that the cholera vibrio might be disseminated on the surface of fruits and vegetables.

He moistened the surface of a ripe apple with some drops of liquid from a cholera stool which dried within 15 minutes. Then after 5, 10, and 20 hours he transferred small particles of this contaminated skin direct to gelatin roll cultures and to tubes of bouillon for 24 hours at 35° C., after which gelatin rolls were made. In each case numerous colonies were obtained. After 24 hours, however, only a few colonies were demonstrable, and after 30 hours, very few. After 48 hours no colonies were obtained. From the surface of an apple treated in the same way except that it was kept under a bell jar, cholera bacilli were demonstrated up to the end of the fourth day.

Similar experiments were performed on the leaf-stalk of a cauliflower. Two infections were made, one (I) on the exposed base of the petiole, the other (II) on the midrib where the blade of the leaf bent over so as to protect the spot from rapid drying.

The spot (I) which was still somewhat moist, yielded numerous colonies after 24 hours, but none at the end of 48 hours, when it was fully dry. The spot II was tested at the end of 24, 48, 72, and 96 hours. Living cholera bacilli were present on it at the end of 24, 48, and 72 hours. They were not demonstrated at the end of 96 hours, and were sparingly present at the end of 72 hours.

The experiments of Wurtz and Bourges (1901) were undertaken to determine whether the culture of certain vegetables should be forbidden on the sewage fields of Paris. Their technique was as follows:

Pots were filled with soil and sowed with seeds of cress, lettuce, and radish. Immediately after, the earth was watered with suspensions of the given microbe, taken from agar cultures or potato cultures. Ordinary plate cultures gave no positive results, owing to the prodigious number of colonies developing from soil bacteria present on the stems. Only in case of three pathogenic bacteria did they obtain positive results from the tips of the leaves by the use of special methods.

In three series of experiments with anthrax they obtained cultures from the leaves by first heating them for 3 minutes at 80° C., and then making gelatin plates. The anthrax organism was recovered constantly and easily up to 3 weeks from the time of the sowing.

The typhoid fever organism was recovered by placing the leaves in ordinary carbolated bouillon at 42° C. for 2 days, after which gelatin plates were poured. With pure cultures from these plates agglutination tests were made. The results were positive in 30 cases out of 30.

The tubercle organism was detected by inoculating into guinea-pigs fragments of leaves bruised in sterile water. The first series gave 1 positive result out of 4. The second gave 18 positive results out of 30.

Experiments with the colon bacillus miscarried.

These experiments were carried on in the laboratory, at room temperature. The plants were exposed to the sun only a short time each day, and were not washed by rainfall. Consequently:

Il y a en effet du danger à faire ces expériences portant sur des microbes pathogènes en pleine terre.

To obtain conditions more nearly approximating those found in the fields they experimented with *Bacterium violaceum* and the red Kiel bacillus.

Seeds of radish were sowed in the open field, were covered with about 2 cm. of earth and were watered with a culture of *Bact. violaceum* suspended in sterilized water. It was at this time very warm and dry. The radishes were watered very abundantly by showering with ordinary water. Fifteen days after the sowing the plants were lifted and a search made for the bacillus, but it could not be found either on the surface of the plants or in the earth where it had been put.

The same experiment was repeated using the Kiel bacillus, but dividing the field into two parts, one of which was shaded, and the other exposed to the sun. The weather continued very hot and the artificial waterings were abundant. The Kiel bacillus was recovered on plate cultures from the surface of the plants grown in the shade, but not from those exposed to the sun, so that we must assume either that the sun destroyed the bacteria, or did away with their pigment production. In the above experiments [probably those with the pigment-forming bacteria] the plants were exposed to the infectious water after they had begun to develop.

To test the ability of the plant to bring up bacteria out of the depths of the soil they made the following experiments.

Potato tubers were wet on the upper surface with a virulent, sporulating bouillon culture of the anthrax organism (about one culture for each three potatoes). They were then placed in boxes of earth and covered with from 5 to 10 cm. of vegetable earth. From March 21 to July 1 these potatoes were watered with large quantities of water, as well before as after the development of the shoots. During growth the plants were watered several times a week. In May and June the plants were exposed to sunshine from morning until 3 p. m.

Fragments of the leaves and stems were taken with sterile instruments and washed in bouillon which was then heated for 5 minutes at 80° C. This bouillon was then used for gelatin plates, 10 drops being put into each plate. In this way the anthrax organism was recovered 41, 93, and 101 days after the sowing, and at heights of from 4 to 30 cm. above the earth. The anthrax organism was constantly present, but usually only a few colonies developed, on an average 1 or 2 per plate. At the end of 3 months these anthrax spores had lost half their virulence.

To reassure themselves of this and to do away with the effect of other soil organisms (*Vibrio septique*, tetanus, etc.) this experiment was repeated in sterile soil. Anthrax colonies taken from the tops of the stems of potatoes grown in this soil had lost their virulence.

In spite of the action of light and of rainfall, there is, therefore, according to Wurtz and Bourges some reason for suspecting vegetables grown on lands devoted to the purification of sewage, even though theoretically the sewage be not deposited in a sheet on the surface of the soil, since plants, as well as animals, may serve to bring pathogenic bacteria to the surface of the earth.

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## HYGIENE OF PLANTS.

Nothing perhaps comes out plainer in a study of diseases of plants, bacterial diseases included, than the fact that such diseases are very often introduced on species or varieties brought in from other localities. No one, for example, would care to buy seed-potatoes from a field subject to brown rot or basal stem-rot, cabbage-seed from plants affected by black rot, sweet corn seed from fields subject to Stewart's disease, olive trees with olive-knot, pear-trees with pear-blight, plum-trees carrying with them the black spot organism, or peach-trees grown in nurseries subject to crown-gall, and yet without knowing it planters are doing these things all the time.

Viewed in this light, the introduction of new things from all sorts of places is not an unmixed good. Many diseases are spread in this way. Especially is the importation in bulk and the immediate general distribution of all sorts of seeds and plants to be deprecated. It would be much safer to import seeds and plants in small quantities and multiply them for a year or two under strict Government supervision in experiment gardens before making a general distribution. The dissemination of many scales and other injurious insects would also be prevented by this method. Up to this time, with local exceptions, *e.g.*, France, Germany, California, growers in all parts of the world have been allowed to import and distribute at will. The growers in the United States in particular do almost exactly as they please. The Department of Agriculture also has sometimes imported and distributed without proper inspection. The machinery of inspection is not properly organized in this country. To do such inspection thoroughly would require a small army of trained inspectors (entomologists and pathologists) distributed at at least a dozen different ports of entry, all subject to one efficient central inspection bureau. Universities are not turning out men fast enough to meet the demands for this sort of work, and at the present time there are not men available in this country to carry out properly any such system of inspection, important as it is to have it instituted speedily, nor even to meet the ordinary requirements of pathological research.

As time goes on undoubtedly strict inspections will be required in all highly civilized countries, and the propagators and distributors of trees, shrubs, herbs, tubers, bulbs, seeds, etc., will be required to give some sort of guarantee as to where the plants were grown and under what conditions. Then seedsmen will not be permitted to sell seeds raised in infected districts and often harvested from infected plants, simply because it is convenient for them to do so, nor will nurserymen be allowed to sell stock known to be infected, for no better reason than simply because they have a large quantity on hand and wish to dispose of it. Meanwhile, in the absence of proper inspections, intelligent buyers will deal only with reliable firms, and will in addition seek for some specific assurance as to healthfulness. In case of large orders a visit to the plantations themselves before the trees are dug, or the seeds harvested, might occasionally prevent much subsequent vexation and loss.

Some propagators of seeds and plants appear to be entirely indifferent to the welfare of the community, sometimes distributing things known to be infected, and there should be a severe law for such people.\* The greater number, however, undoubtedly trespass through ignorance, and we can not hope for a general improvement of the seed and plant trade in these particulars until a knowledge of these diseases, particularly information as to their dangerous nature and the exact methods of their dissemination, is broadcasted through the trade, and made effective through the demands of the buyers for healthy stock. The buyer must be on his guard continually. He knows the undesirability of cocklebur, ragweed, and thistles, but in most cases he has not yet come to realize the greater undesirability of

\*The San José scale was disseminated through the eastern United States by nurserymen of this type.



these microscopic pests. He should write them down in his list of "worst weeds," and try to keep his fields free from them. There ought also to be some legal means of redress when a nurseryman or seedsman has taken hard-earned money and in return has infected a man's land and rendered his business unprofitable, but in most cases there is not. The ounce of prevention, therefore, is the thing to be thought of, and more and more the farmer must consider the advisability of suitably disinfecting trees and seeds before planting them, unless he knows their source to be a safe one. Many dealers who own propagating farms also buy large quantities of stock from other growers, so that the farmer seldom knows from what part of the country his plants have come. He may think he is buying from an uninfected region while in reality his trees may have come from diseased localities. In case of two kinds of seed sold extensively by the trade, *viz.*, sweet corn and cabbage, it is notorious that they are propagated for seed largely in districts where no such seed should be grown, because the plantations often reek with disease, and the germs of these diseases (Stewart's disease of corn and the black-rot of cabbage) are liable to be distributed on the seeds.

Excess of water undoubtedly renders many plants more susceptible to bacterial diseases. The evidence here is very good in a number of cases. It is now a well-known fact first observed, I think, by L. R. Jones, working in the writer's laboratory, that the soft-rot organisms need tissues filled with water in order to make rapid progress. In pear-blight slow growth is favorable to freedom from the disease and excessive moisture leading to rapid growth renders the plant much more susceptible to disease: This has been observed over and over again in many localities. Russell obtained black-rot of cabbage more readily on well watered plots. Halsted observed the same thing in beans. The writer has seen the same thing in sweet corn and in tomatoes. It is, therefore, desirable that moist soil should be properly underdrained and that irrigation should be managed with great care. Lack of subsoil drainage is very favorable to the development of root rots of all sorts especially in rainy seasons. Not infrequently an entire field of potatoes rots within a week from this cause. Excess of water on the foliage also leads to numerous infections as in the case of black spot of the plum, bean-spot, begonia-spot, etc. Where the plants are out of doors this can not be avoided, altogether, but under glass it can be modified by care in throwing water and by so arranging the houses that all parts shall have proper ventilation. Diseases often begin in ill-ventilated parts of the hothouse.

Overcrowding may also be a cause of disease in some instances, especially if it leads to imperfect ventilation and the persistence of water upon the foliage.

In one instance the writer observed a bacterial rot to be favored by excess of shade, namely the iris rot. Under his observation this was very serious one year in heavily shaded parts of a garden, and not at all present on the same grounds in clumps of the same iris exposed fully to the sun. Slow evaporation of water was probably the predisposing cause.

Excess of manures, especially of those containing nitrogen, renders the plant more susceptible to cold and probably also to disease by throwing it out of physiological balance but I can cite no specific instances of bacterial diseases particularly favored by this in any way other than by the formation of rapidly-growing juicy tissues. Infected manures are to be avoided carefully. By this I mean barnyard manures with which diseased plants have been mixed. The writer saw a field of cabbage in Michigan, a small portion of which was much worse diseased by the black-rot than the remainder. This portion contained perhaps an acre or two, and the only difference between it and the remainder of the field appeared to be that it had received as a manure the refuse from a neighboring cabbage storehouse, in which heads affected by the black-rot had been placed the preceding fall. The potato-rots are diseases likely to be transmitted in this way since diseased potatoes are often fed to stock or thrown out on manure piles. Stewart's disease of sweet corn is another disease likely to be distributed in this way since the farmer often feeds the stalks to cattle, and these are swarming with the organism which causes the disease. The bacterial spot of beans is another.

Negligent pruning in some instances may be responsible for the distribution of disease. There is not much doubt that pear-blight may be distributed from one tree to another by means of infected pruning shears. Since this sentence was written D. H. Jones has done it with a pruning saw. Probably olive-tubercle can be spread in the same way: The Italians think so. Apple gall is favored by carelessly made grafts (Hedgcock). Daisy knot frequently appears on the wounded end of cuttings.

The wounding of the roots of plants in transplanting from the seedbed to the field is a fertile source of infection. The most striking examples of this are the distribution of tobacco wilt and of tomato wilt. It would be best, if possible, to avoid transplanting into fields subject to these diseases. If such fields must be used, then the seeds should be planted where the plants are to be grown or the transplantings made with unusual care. Any wounding of the roots in such soils is highly detrimental because the plants seem to be able to keep out the soil organism so long as the roots are not broken, but when these are injured there is an open passageway into the vascular system of the plant which the parasite is not slow to find. It has been observed (Hunger) that this wilt disease is worst on fields occupied by plant-infesting nematodes, the direct loss due to these worms being a small part only of the actual injury since the wounds they make form an open passageway through which the bacteria enter the plants and destroy them. A great desideratum is some easy means of combating in the soil this type of nematodes. The early removal and destruction of trap crops is supposed to be a partial remedy. On small areas, *e. g.*, in houses or under tents, they may be destroyed by the use of steam.

Having said thus much about nematodes it remains to say a word about the destruction of insect carriers of disease. We know, from Mr. Merton B. Waite's experiments, that pear-blight is commonly distributed by bees and flies. My own experiments have, I think, settled the fact that the wilt of cucurbits is distributed by *Diabrotica vittata*. Brenner plated *Bact. campestris* from an aphid allowed to puncture diseased veins of a cabbage-leaf. Jones has recently plated *Bacillus amylovorus* from aphides feeding on blighting shoots of the apple. The question is yet, perhaps, an open one whether aphides are responsible for the general distribution of many bacterial diseases. The same is true for various other bugs which have been incriminated, *e. g.*, the squash-bug. Exact experiments are still wanting.

On general principles, however, it is desirable to keep down the prevalence of biting and puncturing insects both in hothouse and field by the use of suitable insecticides.

Change of crops is an important means of combating many diseases. This is particularly true of bacterial diseases if we may accept Laurent's idea as in any degree representing what really takes place in the soil. His laboratory experiments led him to believe that non-parasitic forms are often converted into transitory parasites by change of food, *i. e.*, by growing for a time saprophytically on portions of the plant they become able to attack it parasitically. If, on the contrary, the organisms which have attacked plants are compelled to live for a time on other foods they lose the parasitic habit, *i. e.*, become non-virulent. If this is true, fields which have developed a disease should be plowed so as to aerate the soil and thus hasten the decay of diseased roots and stems. Moreover, if possible the field should be put to other crops for some years. Rotation of crops is an old subject much written upon, but not yet sufficiently impressed upon the multitude of planters. To follow one crop with another of the same sort and that year after year invites disaster.

We do not yet know much about the proper chemical treatment of soils to put them into the best condition to resist a particular disease, *i. e.*, use of lime, acid phosphates, etc., but there is undoubtedly much to be learned. Of this fact I think there can be little doubt, namely, that bacterial plant parasites die out of some soils more readily than out of others.

This chapter may well close with a pregnant sentence from Louis Pasteur:

Il est au pouvoir de l'homme de faire disparaître de la surface du globe les maladies parasitaires.

## RECOVERY BY EXCISION.

It would be difficult to say when excisions were first practiced for the protection of plants. The method is probably as old as gardening itself. It is a common practice in China. It has been, however, purely empirical.

The systematic practice of excision of plant-parts with a particular end in view, that end based on a scientific knowledge of the habits of the parasite to be overcome, is comparatively modern.

Wakker seems to have been the first scientific man to try this for a bacterial disease of plants. Knowing that the movement of the bacteria was downward in the vessels, he removed the leaves of hyacinths showing the beginnings of bacterial disease at their apex, and thus prevented the infection of the bulbs.

Dr. Russell and the writer both showed that infection of cabbage-heads by *Bacterium campestris* could be prevented by removal of leaves only recently out of the water-pore stage of infection, the movement of the bacteria in this case also being downward.

For details on excision experiments made by the writer to control wilt of cucurbits, see page 276. By the very prompt removal of affected leaves or branches valuable plants may sometimes be saved, but usually the wilt is not discovered until the bacillus has entered the main stem and then excisions are of no avail.

The most successful example of protection by pruning known to the writer is the winter treatment of fire-blight of the pear, devised by Merton B. Waite. Mr. Waite discovered that *Bacillus amylovorus* winters over in pear-trees, but only in a small proportion of the whole number of the trees attacked, one tree, let us say, in fifty, or one in a hundred, the exact proportion is immaterial. From the gummy exudate out of patches of bark on these trees, through the agency of insect-carriers (bees, flies, etc.) the bacteria are distributed again the following spring to other trees in the orchard and the blight renewed—first, usually, as blossom-blight. Mr. Waite has not been able to find any other source of the early spring infection. These cases of "hold over blight," as he has called them, can be detected by sharp eyes and removed while they are in a dormant condition; and, theoretically at least, if all were thus removed, there would be no source of infection the following spring, and consequently no new blight. The correctness of this hypothesis was first tested on a considerable scale in Georgia, where Mr. Waite succeeded in saving a large pear-orchard which had been threatened with complete destruction. Every visible vestige of the winter-blight was removed from these pear-trees several years in succession, and the disease ceased to be a cause of anxiety. In fact, the only cases that appeared thereafter in this orchard were scattering examples traceable to an occasional case of hold-over blight which was missed during the winter examinations, and to such infections as wandered into the borders of the orchard from the small neglected orchards of careless neighbors. The experiment was considered to be a brilliant success, both by the pathologist and by the owner of the orchard.

In more recent years Mr. Waite and his assistants have endeavored to apply this method on a large scale to the pear-orchards of California. When he was urged to undertake the task, about one-fourth of the orchards of the State had already been destroyed by this disease, and the remainder were threatened with destruction, the disease being widely disseminated, although of recent appearance, and very aggressive. To be asked to do for a whole State what he had done before for a single large orchard might well appal any man. The loss of these orchards meant, however, a money loss to the State of California of perhaps ten million dollars. Mr. Waite, therefore, energetically undertook to save them. The task, however, was a gigantic one since it involved critical inspection of every pear-tree in the State, and the removal of all diseased parts during the winter season. It involved also the education of the pear-growers of a whole State, the combating of much ignorance,

the persuasion of many obstinate, doubtful individuals. For this purpose not enough money was available. As a result of the first year's work (winter of 1905-6) the disease was checked in many places, but the surgeons were too few and the cooperation of all the growers could not be obtained. The blight, therefore, still prevailed in many places. In succeeding years, by more vigorous efforts and a general campaign of education, many of the more intelligent pear-growers now assisting in the work of eradication, the disease has been so restricted as to afford a further demonstration of the immense value of systematically conducted winter-excisions.\* Mr. O'Gara has carried on a similar campaign of eradication against blight in the apple and pear orchards of Oregon (Rogue river valley) with the very best results in the protection of the magnificent orchards which there also were threatened with destruction. Even in localities in California where practically everything has been swept away by blight single growers of great energy and intelligence have saved their pear orchards by systematic removal of blighting wood. The pear orchard of 6,000 trees owned by Mr. Reed at Marysville is a good example. This is now almost or quite the only orchard of any size left in that region, the others having been destroyed by the blight.

It is not too much to say, therefore, that excision is a remedy of prime importance in case of pear-blight, and that the disease can be held in check thereby. The principal difficulty lies in obtaining that unity of action throughout a community which is absolutely essential to full success.

In case of the olive-tubercle equally vigorous treatment would probably yield equally good results. This statement is based on hothouse observations of inoculated plants metastasizing freely. In 6 or 8 months from the first appearance of a primary tubercle the organism causing the disease may pass downward through the stem in young and vigorous plants a distance of 18 to 36 inches or more, rupturing to the surface at intervals in the form of deep seated secondary tubercles. It is therefore of the utmost importance for the removal of this disease to prune early and severely so as to reach beyond the unseen advancing bacteria.

### GERMICIDES AND INSECTICIDES.

The surface sterilization of plants or parts of plants has been treated in Vol. I, so far as regards its use in connection with the isolation of pure cultures. To what has been said there may be added some remarks on the newer germicides and on the reasons for the occasional ineffectiveness of mercuric chloride. Respecting the latter it is a well-known fact that organic substances of various sorts throw this salt out of solution fixing it in their outer layers. Consequently in many cases its *penetrating power* is slight, and even when a rather strong solution is used the deeper layers of a substance may not be reached and consequently some bacteria may escape. For this very reason it is well adapted to the surface disinfection of thin tissues such as leaves from the interior of which we wish subsequently to make a culture. The exposure, however, must be very short.

It has also been shown in recent years that a killing quantity of mercuric chloride may be rendered ineffective by a subsequent soaking of the treated bacteria in water, *i.e.*, the poison can be washed out of the outer layers of the bacterial membrane and sometimes growth will then take place. These facts must be borne in mind whenever mercuric chloride is used. The writer still uses it as described in Vol. I. It is not likely that it always fully sterilizes the surfaces treated, but long experience has shown that it does do so to an

\*In a letter dated June 6, 1908, from Auburn, California, Mr. P. J. O'Gara wrote to me as follows:

"The work in California on pear-blight control is showing excellent results wherever there has been united effort in eradication. To show you how hard it is for me to get a culture of the organism, I traveled all one day through the best pear section of Placer County and only succeeded in getting one case. Another interesting fact is that there are more pear trees growing in this county (Placer) and adjoining counties than there were three years ago. The local nurseries have not been able to supply the demand during the past year or two, all of which speaks very well for our office."

extent sufficient to exclude rapid-growing surface organisms from participation in the subsequent poured-plate cultures, so that pure growths can be obtained without difficulty from deeper portions of the plants.

So far as known to the writer no serious attempts have been made to try the newer germicides on plant parasites. A very few of these may be mentioned. Atoxyl, known chemically as arsenic-plate-acid anilide, or arsanilic acid, has been used during the last few years for injection into the spinal cord in cases of sleeping sickness with a view to destroying the trypanosomes. Statements respecting its value are conflicting with the preponderance of the evidence in favor of the use of the germicide. It is undoubtedly only a makeshift till something better is found. Atoxyl is much less poisonous to the higher animals than white arsenic, but very deadly to some of the lower forms. Some of the more recently introduced related substances such as atoxyl acetyl or arsacetine, and especially arsenophenylglycin, or arsenophenylglycollate of sodium, are still more efficient against trypanosomes and at the same time less poisonous to the higher animals (mice, rats, guinea pigs, rabbits, dogs, horses). This latter salt, which, according to Ehrlich, is a little less toxic than atoxyl (but two to four times less toxic according to Roehl) is said to have a very high therapeutic value. A single dose is said to have cured an animal suffering from an experimental tripanosomiasis.

The most-talked-about substance at present is the dioxydiamidoarsenobenzol from Ehrlich's laboratory, commonly called No. 606 and also salvarsan. A single dose of this substance if properly administered is said to be sufficient to destroy every vestige of *Treponema pallida* in the body of a man. But here again conflicting statements are rife (1911).

Argyrol is an organic silver salt now much used by oculists and others. There are various other similar silver compounds possessing some of the germicidal properties of nitrate of silver without its caustic properties, *e. g.*, protargol, argonin, albargin, largin, saphol (formaldehyd, nucleinic acid and silver). Some of these organic silver compounds are strongly germicidal without being very injurious to the animal body, *e. g.*, argonin, argyrol, albargin.

Persistent claims have been made by the manufacturers for the high disinfectant power of cyllin, and some of these claims appear to be borne out by scientific tests. Cyllin appears to be some sort of a phenol or mixture of phenols. It is said to be much less poisonous than carbolic acid, and twenty times as efficient, *i. e.*, nearly or quite as germicidal as mercuric chloride. It is advertised in reputable journals as "non-toxic."

Aniodol, a French disinfectant, has been recommended recently as a substitute for iodoform. It is said to be highly germicidal and may be used as a powder or as a soap. It contains neither mercury nor copper.

Cook's asepto soap has been recommended by the Journal of Tropical Medicine. This contains 3 per cent biniodide of mercury. One gram of the soap in 60 cc. of water is said to be equivalent to 1:2000 of the biniodide of mercury, and the lather of the soap is supposed to be still more effective. It is said to be a remedy for Favus.

For notes on the newer germicides see back volumes of the Journal of Tropical Medicine and Hygiene, Bulletin de l'Institut Pasteur, and New and Non Official Remedies, 1911, Press Am. Med. Asso., Chicago.

The writer made several tests of the St. Lâcleau soap (see Vol. I, p. 253) using spores of *Bacillus subtilis*. It appeared to be without sensible restraining effect on this organism, *i. e.*, *B. subtilis* grew readily in bouillon after exposure to 10 per cent solutions for 30 minutes, and that, too, without a preliminary washing of the spores. Even when the soap was added to agar in the proportions recommended by Kourâdi it did not prevent the growth of bacteria. The particular cake tested had been out of the factory three years, but had been recently removed from the box and original tinfoil wrappings and was unchanged in appearance.

The absolute sterilization of the surface to prevent the plant from contracting disease, to prevent infection of the soil by suspected seeds, cuttings, etc., or finally to prevent the infection of menstria and the consequent miscarriage of special pathological, physiological, or chemical experiments is sometimes a matter of considerable importance. Unfortunately our knowledge is still very incomplete. Often no specific advice can be given, preliminary experiments being necessary in particular cases. The best that can be done here is to make a few suggestions and record the results obtained in particular instances. The reader is also referred to the special cases mentioned under particular diseases.

For holding solutions sterile for short periods without changing their composition the chemists have used chloroform, thymol, toluene and similar substances. To get the full restraining effect of these substances the material to which they have been added should be shaken continuously and shut away from the air; otherwise bacterial growths are likely to appear (see Vol I, p. 74). Glycerin is not germicidal to *Bacillus subtilis* see p. 149. Continuous exposure at 1° C. or at 80° C. will often answer when the nature of the reaction desired, or of the substance to be isolated, will permit it. The growth of aerobes can be held in check by substituting for air some inert gas such as hydrogen or nitrogen. Surfaces can not be washed free from bacteria.

Inasmuch as pathogenic bacteria are often transmitted from diseased plants to healthy plants by insects, the wholesale destruction of the latter often becomes imperative, and some comments are, therefore, added on the most efficient insecticides.

In 1908, H. Chick and C. J. Martin published a paper on the standardization of disinfectants in which they record the following results.

It is generally recognized that in any method of standardization the temperature, and the composition of the culture medium should be constant, while the number of bacteria per unit volume and the resistance of the test organisms used should be as constant as possible. The authors adopted 20° C. as the temperature most closely approximating the conditions of practical disinfection. Thirty minutes was found the most satisfactory time unit of exposure, as a shorter time was unfavorable to mercury and silver salts, while for phenol and emulsified disinfectants either 10 minutes or 30 minutes was satisfactory.

It was found best to employ a sulphide to neutralize the traces of disinfectant carried over with the test sample. Mercuric chloride required an excess of sulphide to decompose a compound formed between the mercuric salt and the substance of the bacteria which prevented growth. The most satisfactory results were obtained with 0.2 cc. of saturated solution of hydrogen sulphide in distilled water to 10 cc. of broth.

The efficiency of a disinfectant was found to vary with the organism used. In the case of spores, metallic salts were the most effective germicides. These were effective in very small concentration (1000 times less than phenol). With phenol, sporing forms were from 17 to 25 times more resistant than vegetative forms. Virulent strains were generally more resistant than non-virulent ones.

Since in practice disinfectants are commonly used in the presence of organic matter it seemed desirable to introduce this factor into the process of standardization. Experiments with this in view showed that 10 per cent blood serum reduces the efficiency of phenol about 12 per cent. A somewhat greater reduction occurs with emulsified disinfectants and a much greater with mercuric chloride. A solution containing 0.5 per cent of the latter was reduced from 0.6 to 0.06 of its original value as the concentration of serum was increased from 5 to 30 per cent. The presence of particulate organic matter (dust, animal charcoal, finely pulverized coagulated albumen, bacteria and faeces) affects the germicidal value of emulsified disinfectants far more than that of phenol. Commercial cresols were reduced in efficiency 30 to 50 per cent by the introduction of such matter. Finer emulsions are more seriously reduced than coarse ones. This reduction was shown to be principally due to adsorption of the emulsion upon the surfaces of the particles.

In 1908, Dr. Rideal discussed the question of uniform methods of testing disinfectants. He states what every one knows that it is very difficult and in many cases impossible to compare the results of different workers because no definite standards have been adopted. He cites tests of Koch, Esmarch, Fraenkel, Geppert, Klein, Cash, Wynter Blyth, and Sternberg as examples.

Some of Rideal's comments (*Journal of Tropical Medicine*) are as follows:

In 1897 Krönig & Paul recommended substituting small garnets of uniform size for Koch's thread method, since the garnets could be washed and treated with chemical reagents to neutralize the disinfectants. They tested the results of disinfectants quantitatively by making poured plates. Madsen & Nyman (*Zeits. f. Hygiene*, Bd. 57, p. 388) and Miss Chick (*Journal of Hygiene*, February, 1908) have shown that their results can be plotted in curves. These show that the velocity of disinfectants depends primarily on number of bacteria to be destroyed, and secondarily on their resistancy or age.

When anthrax spores are used they may be regarded as of practically the same age and resistance. With ordinary cultures of non-spore-bearing organisms, he states there are individuals of different age having different resistancy, and then the velocity of the disinfectant is not so simple. He says that Miss Chick has shown that by taking cultures of short periods of 3 hours, and sub-culturing, the organisms present in such cultures become uniformly more resistant, and the velocity of disinfection approximates to the same law governing disinfection of spores. Miss Chick has also found that temperature influences the process of disinfection, disinfection being more rapid in warm climates than in cold ones. Dr. Rideal states that Miss Chick's work has confirmed the method of disinfection recommended by Walker & Rideal in 1903. He says the elaborate method of the use of garnets "may be replaced advantageously by the drop method known under our names." They used carbolic acid as the basis of their tests. Next after the question of standardization of the disinfectants, he considers the culture broth of primary importance. The test organisms should be grown for 24 hours and then inoculated into primary test cultures always made up from nutrient broth of a definite composition. Rideal and Walker have suggested that the broth should contain 20 grams of lemco, 20 grams of Witte's peptone, and 10 grams of salt per liter of water, and should after boiling be neutralized with caustic soda, after which 15 cc. of normal hydrochloric acid are added per liter.

"Having secured a standard broth, standard carbolic acid, and a standard culture, the only other conditions are those of temperature and sterility."

Rideal says that these and other details are given in a little book by W. Partridge on the "Bacteriological Examination of Disinfectants," published in 1907 by the Sanitary Publishing Co. He says further:

"In view of the very great development that has taken place in our knowledge of the constitution of the derivatives of phenol, various refined and highly scientific products have been put upon the market by enterprising manufacturers, and it is, therefore, desirable that the medical man may have a weapon by which these products may be identified and classified according to their germicidal efficiency, not only because these newer products are so valuable in preventing and eradicating disease, but in the comparatively new field of medical application for internal treatment, where, of course, the efficiency of the dose is of supreme importance.

"Thus, for example, I understand that Dr. Wright and Dr. Morgan in working on cancer are using this test for determining the germicidal value of cinnamic acid derivatives for internal use whilst Dr. Hartigan, in your own *Journal* in 1905, pointed out that a well known disinfectant with a high coefficient [eyllin, probably] could be used in sprue as an intestinal disinfectant when administered in the form of palatinoids, and Fleet Surgeon MacNab has similarly found that it can also be used internally in treatment of Mediterranean fever. Captain Brodribb has also used the same disinfectant in cantonments in India as a douche for the treatment of gonorrhoea in women."

In a discussion of this paper Dr. Sommerville referred to the necessity of introducing organic matter into the cultures so that the laboratory tests are in a measure conformed to the actual conditions occurring in practice. He says:

"The question of prime importance is the type and quantity of organic matter which should be introduced. From work executed a year and a half ago, it was found that less than 10 per cent of organic matter brought down the coefficients of all disinfectants to the same figure. Mr. Ainslie Walker and I have recently adopted a 1 per cent mixture of starch and gelatin."

Professor Hewlett said:

"I think that all those who have had anything to do with this Rideal-Walker test must agree with the beautiful simplicity of the method, and I think the greatest credit and congratulations are due to Dr. Rideal and Mr. Ainslie Walker for working out the test. It is really the first practical method that has been devised for comparing the germicidal efficiency of disinfectants. \* \* \*

"Lastly there is the point which Dr. Sommerville has raised; the test, though so beautifully efficient for so large a number of disinfectants, lacks, of course, in one point, namely, that one is acting upon naked germs, whereas in actual practice the germs are mixed with organic matter. There is a certain lowering of the efficiency of the disinfectant in the presence of organic matter, so

that we want to increase the efficiency of the test, if we can, by the addition of some form of organic matter, which will aid us in determining the real efficiency of disinfectants in the presence of organic matter. This point is especially important in connection with disinfectants which act by oxidation. If you try permanganate of potash on naked organisms you will find that it is very efficient, but if you mix with it a little dust you will find that its power has largely gone down."

Dr. Schryver in the discussion also cited the physical action of organic matter held in suspension as well as in solution and considered this to be important.

### GERMICIDAL TREATMENT OF SEEDS.

Seeds with a thick impenetrable seed-coat offer no special obstacles to thorough disinfection. All that is necessary is actually to wet every part with a strong germicide for a sufficient length of time. To insure this wetting there should be a brief preliminary wetting in alcohol that air may be driven out of all the minute crevices where otherwise the germicide would not penetrate.

The case is quite different, however, with seeds having delicate and easily permeable seed-coats. These have to be treated with great care, and often it is not possible to disinfect their surface thoroughly without at the same time destroying the embryo, at least in a large proportion of the seeds. Sometimes in such cases we may reach the end desired indirectly, *i. e.*, by not allowing the seeds to become infected, since they are always originally sterile inside the unopened pods. To obtain sterile seeds it is suggested that the unopened seed-pods be collected with great care and their surface treated with germicides or fire, or both, after which the pods must be carefully opened (in still air), and the seeds removed by means of sterile forceps (see fig. 2, and p. 135).

The writer found that 15 minutes' exposure of hard dry kernels of sweet corn to 1:1000 mercuric chloride did not entirely sterilize the surface, although from the results obtained it must have come very near to doing it, so far as regards the death of the organism in question, *i. e.*, *Bacterium stewarti*.

Experiments made by the writer in the summer of 1909 with hybrid dent corns and sweet corns having a high germinating capacity showed that the dry kernels would stand exposure to 1:1000 mercuric chloride water for 20, 30, 40, and 50 minutes with little injury, the kernels being placed at once in damp sand after preliminary rinsing in hydrant water or without rinsing. Nearly all germinated promptly and the seedlings looked as well as those from the untreated seeds.

In the first series twenty seeds were planted in each pot and the following are the number of germinations per pot, the count being made on the sixth day:

- |   |   |
|---|---|
| (1) U. S. P. B. No. 100 (field-corn):               |   |
| Checks, 20, 20, 20, 19, 19;                         | Checks, — none.                                     |
| Mercuric chloride (20 minutes), 18, 20, 17, 19, 19; | Mercuric chloride (30 minutes), 18, 19, 18, 19, 20. |

In a second series of tests, using 20 seeds and counting on the fifth day, the following results were obtained:

- |   |   |
|---|---|
| (2) U. S. P. B. No. 120 (field-corn):               |   |
| Checks, 14, 18, 14, 17, 16;                         | Checks, 16, 15, 17, 16, 18;                         |
| Mercuric chloride (40 minutes), 18, 19, 15, 19, 12; | Mercuric chloride (50 minutes), 18, 17, 16, 18, 20. |

There was no marked difference in the appearance of the seedlings.

The experiments were repeated a few days later with this difference only, that the seeds were not rinsed, but dried promptly and planted at once with the mercuric chloride adhering to them. The results on the sixth day were as follows:

- |   |  |
|---|--|
| (1) U. S. P. B. No. 100 (field-corn):               |  |
| Checks, 18, 20, 20, 19, 20;                         | Checks, 18, 19, 20, 19, 20;                        |
| Mercuric chloride (20 minutes), 19, 18, 16, 19, 17; | Mercuric chloride (30 minutes) 18, 20, 19, 20, 19. |

The treated seeds showed a slight retardation in germination. Checks 2 to 3 inches high; treated 1.5 to 2 inches high.



The experiments were then continued as follows:

- (2) U. S. P. B. No. 120 (field corn):  
 Checks, 20 19, 20, 20, 20;  
 Mercuric chloride (40 minutes), 18, 18, 13, 18, 15;  
 Checks, 20, 18, 19, 18, 20;  
 Mercuric chloride (50 minutes), 16, 18, 16, 18, 15.

The most injury (retardation) was in seeds exposed 40 and 50 minutes. A few were just coming up on the sixth day.

- (3) Yellow Dent.—Examined at the end of seven days:  
 Checks, 20, 20, 20, 20, 20,  
 Mercuric chloride (20 minutes), 20, 20, 20, 20, 20.  
 Checks, 20, 20, 20, 20, 20;  
 Mercuric chloride (30 minutes) 20, 20, 20, 20, 20.  
 Checks, 20, 20, 20, 20, 20;  
 Mercuric chloride (40 minutes), 19, 20, 20, 20, 20.  
 Checks, 20, 20, 20, 20, 20;  
 Mercuric chloride (50 minutes), 20, 20, 20, 20, 20.

Considerable retardation at first, especially in longer exposures. Scarcely any in the 20 minute exposure at end of experiment, and all making a good growth.

- (4) White Flint.—Examined at the end of eight days:  
 Checks, 20, 20, 20, 20, 20;  
 Mercuric chloride (20 minutes), 20, 20, 20, 20, 20.  
 Checks, 20, 20, 20, 19, 20;  
 Mercuric chloride (30 minutes), 19, 20, 19, 20, 20.  
 Checks, 19, 20, 20, 20, 20;  
 Mercuric chloride (40 minutes), 20, 20, 20, 20, 19.  
 Checks, 19, 20, 20, 20, 19;  
 Mercuric chloride (50 minutes) 19, 20, 19, 20, 20,

Considerable retardation at first, especially in longer exposures. Scarcely any in the 20 minute exposure at end of experiment, and all making a good growth, even the plants from seed exposed for 50 minutes.

- (5) Black Mexican, examined at the end of 7 days:  
 Checks, 19, 17, 19, 18, 18.  
 Mercuric chloride (20 minutes), 16, 18, 18, 19, 17.  
 Checks, 17, 17, 19, 20, 19,  
 Mercuric chloride (30 minutes), 19, 16, 19, 19, 17.  
 Checks, 20, 18, 15, 20, 16.  
 Mercuric chloride (40 minutes), 19, 19, 18, 18.  
 Checks, 16, 18, 18, 15, 19.  
 Mercuric chloride (50 minutes), 19, 18, 17, 18, 18.
- (6) Early Evergreen, examined at the end of 8 days:  
 Checks, 18, 18, 19, 19, 20.  
 Mercuric chloride (20 minutes), 18, 19, 18, 20, 19.  
 Checks, 17, 20, 20, 18, 20.  
 Mercuric chloride (30 minutes), 18, 19, 17, 19, 19.  
 Checks, 19, 19, 18, 20, 20,  
 Mercuric chloride (40 minutes), 18, 19, 19, 18, 17.  
 Checks, 20, 19, 18, 19, 18.  
 Mercuric chloride (50 minutes), 20, 19, 20, 16, 18.
- (7) Country Gentleman, examined at the end of 8 days:  
 Checks, 19, 19, 18, 19, 18.  
 Mercuric chloride (20 minutes), 19, 20, 19, 19, 19.  
 Checks, 16, 18, 19, 18, 20.  
 Mercuric chloride (30 minutes), 20, 20, 19, 19, 18.  
 Checks, 20, 18, 20, 19, 19.  
 Mercuric chloride (40 minutes), 20, 20, 20, 18, 20.  
 Checks, 19, 20, 18, 19, 20.  
 Mercuric chloride (50 minutes), 20, 18, 19, 17, 20.

The retardation was about the same in 5, 6, and 7. It was most in those exposed for the longest periods. There was no serious injury in any. All made good plants.

- (8) Old Colony, examined at the end of 7 days:  
 Checks, 19, 19, 18, 19, 20.  
 Mercuric chloride (20 minutes), 20, 19, 19, 20, 19.  
 Checks, 20, 18, 20, 17, 19.  
 Mercuric chloride (30 minutes), 17, 19, 17, 17, 20.  
 Checks, 18, 19, 19, 19, 19.  
 Mercuric chloride (40 minutes), 18, 20, 19, 18, 19.  
 Checks, 17, 17, 17, 20, 19.  
 Mercuric chloride (50 minutes), 18, 16, 18, 17, 19.
- (9) Country Gentleman (another source), examined at the end of 7 days:  
 Checks, 19, 19, 18, 19, 19.  
 Mercuric chloride (20 minutes), 20, 19, 19, 18, 20.  
 Checks, 18, 20, 19, 19, 20.  
 Mercuric chloride (30 minutes), 19, 18, 20, 19, 19.  
 Checks, 20, 18, 19, 19, 17.  
 Mercuric chloride (40 minutes), 16, 16, 18, 20, 18.  
 Checks, 20, 20, 18, 19, 19.  
 Mercuric chloride (50 minutes), 19, 18, 15, 20, 18.

Plain retardation in treated seeds of 8 and 9, especially the longer exposures, but all growing well when removed.

- (10) Golden Bantam, examined at the end of 7 days:  
 Checks, 15, 15, 13, 13, 16.  
 Mercuric chloride (20 minutes) 12, 16, 16, 14, 16.  
 Checks, 16, 13, 14, 17, 14.  
 Mercuric chloride (30 minutes), 19, 16, 16, 15, 18.  
 Checks, 17, 16, 15, 8, 14.  
 Mercuric chloride (40 minutes), 18, 18, 16, 16, 16.  
 Checks, 14, 14, 12, 17, 17.  
 Mercuric chloride (50 minutes), 10, 17, 14, 15, 17.

Very little retardation of this variety in 20, 30, or 40 minutes' exposure. In all cases 100 cc. of the 1:1000 mercuric chloride water was used for each 100 seeds.

Many experiments have been made with grains to free them from smut fungi and in this way considerable knowledge has been gained respecting the toleration of seed wheat, oats, etc., for hot water, copper salts and various other disinfectants. Some of the leading papers are mentioned under Literature. Only a few of the results will be cited here.

Jensen who discovered the hot-water treatment for stinking smut (1888) advised temperatures between 127° and 133° F., and exposures of seed wheat and oats for not over 5 minutes. He did not, however, determine accurately the thing we are here specially interested in bringing out, namely, the killing temperature for the grains.

In 1890, Arthur determined the effect of hot water on the germination of wheat. Wheat seeds immersed 5 minutes in water at 135° F. (57° C.) are not injured. Six hundred seeds exposed to 130° F. (54° C.) for 10 minutes also gave excellent results on germination—12.5 per cent in 24 hours and 93 per cent in 5 days. The injury to those treated 10 minutes at 135° F., and 5 minutes at 140° F. (60° C.) equaled about 20 per cent. The limit of germination is 150° F. for 5 minutes (33 per cent). No germinations were obtained when wheat seeds were exposed to higher temperatures, *e. g.*, 155° F. for 5 minutes, or to 150° F. for 10 minutes.

In 1891 Arthur tested the effect of hot water on oats, with especial reference to the prevention of loose smut. He states that the hot-water treatment—10 minutes in water at 135° F., or 5 minutes in water at 135° F. to 140° F. (57° C. to 60° C.) entirely destroys the smut while at the same time it improves the growth and increases the yield of oats. The water may be even as hot as 145° F. when the oats are first put into it without much injuring the germination. Arthur made the exposures in cheese-cloth bags.

Latta found 5 minutes' exposure of oats in copper sulphate water (1 pound to 1 gallon) destroyed the smut but the germination was slower and the yield per acre was reduced. The comparative yields were: Hot water, 33 bushels; untreated, 28 bushels; coppered, 24 bushels. Arthur, who reports this, tested the effect of copper sulphate on germination on lots of 200 seeds and obtained in the germinating chamber the following per cents: Hot water, 99; untreated, 98; copper sulphate, 67. Even in the soil where 98 per cent of the oats treated with copper sulphate finally germinated, they did so very slowly, the primary roots were often killed, and often they pushed out the plumule in advance of the roots.

Kellerman & Swingle (1890) found that exposure of wheat at 139° to 140° F. for 15 minutes destroyed nearly all of the kernels, *i. e.*, on a plot that should have yielded 3,000 or more heads there were only 9. Copper sulphate 8 per cent, 24 hours, limed or unlimed, reduced the germinations about one-fourth. Copper sulphate 5 per cent 24 hours, unlimed, reduced the yield nearly one-third. Bordeaux mixture reduced the yield over one-fourth. Eau celeste, 24 hours (on another page the time is said to have been 36 hours) destroyed all. Carbolic acid 5 and 10 per cent for 20 hours destroyed all. Mercuric chloride 1 per cent for 20 hours destroyed all. Potassium bichromate 5 per cent for 20 hours destroyed about half.

According to Kellerman & Swingle (1891) oats which were treated at 141.8° F. (61° C.) for 5 minutes gave a good crop. The same result was obtained by exposing at 138.2° F. (59° C.) for 10 and for 15 minutes, *i. e.*, there was no destruction of the seed. Potassium sulphide 0.75 per cent and 0.5 per cent for 24 hours reduced the number of stalks about one-fourth. Copper sulphate 0.1 per cent for 24 hours reduced the number of heads about one-fourth. Copper sulphate 0.5 per cent for 24 hours reduced the number of heads nearly half. Copper nitrate in 5 per cent solution for 24 hours, limed or unlimed, destroyed most of the seeds. Even 2.5 per cent or 1 per cent greatly reduced the crop. Corrosive sublimate 0.1 per cent for 24 hours reduced the yield three-fourths. Potassium bichromate 10 per cent for 23 hours killed all; same, 1 per cent for 9 hours, reduced the crop one-half or more.

They recommend treating oats for smut by (1) hot water: temp. 132.5° F., time 15 minutes; or, (2) potassium sulphide: 1 pound to 20 gallons of water, time 24 hours.

In 1891, Kellerman Sr., recommended hot water over all other fungicides for great efficiency without injury to seeds. His experiments were with wheat kernels for the prevention of stinking smut. As a result of many experiments (about 70) he recommends exposure for 15 minutes to a temperature of 131° F. (55° C.). Hot water for 5 minutes at 137° F. and at 138° F. (seeds previously soaked 10 hours) destroyed all the grains. The following treatments greatly injured or nearly or quite destroyed the grains: Bordeaux, 24 hours; same, half strength; 1 per cent copper sulphate, 24 hours; 1 per cent copper acetate, 24 hours; 1 per cent copper chloride, 24 hours; 1 per cent potassium bichromate, 24 hours. The following treatments gave reasonably good yields, *i.e.*, better than the checks, but not as good as the hot water: Copper sulphate 0.5 per cent, 24 hours, limed; copper sulphate 0.5 per cent, 12 hours, limed; copper acetate 0.5 per cent, 24 hours; copper nitrate 1.0 per cent, 24 hours; copper nitrate 0.5 per cent, 24 hours; mercuric chloride 0.05 per cent, 24 hours. The following gave a yield nearly equal to the checks: Eau celeste, 24 hours; mercuric chloride, 0.1 per cent, 24 hours. Ratio of grain to volume of fluid not given. Hot water at 136° F. (57.7° C.) for 5 minutes, then quickly cooled, appears to be the severest exposure compatible with a good crop.

In 1893, Hitchcock and Carleton published the results of their experiments with maize. They tested the effect on germination of 82 chemicals in various strengths, making a total of 400 experiments.

They obtained in moist sand a germination of 80 to 100 per cent (retarded) after exposure to the following strengths of mercuric chloride water: 0.1 per cent for 1, 3, 5, 8, hours; 1.0 per cent for 1 hour. One per cent mercuric chloride for 24 hours or 3 per cent for 1 hour killed all. Chromic acid 1 per cent for 48 hours gave 75 per cent retarded germinations. Copper chloride 10 per cent for 24 hours gave 100 per cent retarded germinations. Copper nitrate 10 per cent for 24 hours gave about 80 per cent retarded germinations. Potassium permanganate 2.5 per cent for 24 hours gave 100 per cent germinations. Hyposulphite of soda 10 per cent for 24 hours gave full germinations. From 80 to 100 per cent of retarded germinations were obtained after exposure to potassium cyanide as follows: 1 per cent, 1 hour; 5 per cent, 1 and 3 hours; 10 per cent, 1 hour. The same, 0.5 per cent for 1 hour scarcely affected germination.

In 1897, Bolley published studies on the fungicidal treatment of wheat, oats and barley which he had carried on for a period of 5 years. The following are some of his conclusions respecting resistance of the dry grain. He states that if the wheat grain is dried at once germination is not retarded by applying corrosive sublimate solutions in strengths up to 4 parts in 1,000 parts of water: Of selected seed of Scotch Fife wheat exposed 2 minutes, 95 per cent germinated; exposed 3 minutes, 82 per cent germinated; 4 minutes, 72 per cent; 5 minutes, 78 per cent; 6 minutes, 67 per cent; 7 minutes, 45 per cent; 20 minutes, 17 per cent; 25 minutes, 0. As little as 0.1 per cent corrosive sublimate weakened the first growth in a rapidly increasing degree in exposures longer than 3 minutes, but even from too strong treatments the final after-growth is stronger than from untreated grain. Mixed samples of oats treated with 0.3 per cent corrosive sublimate water for 30 minutes gave good first growth (94 to 100 per cent germinations) and a good yield per acre. Barley after 15 minutes exposure to 0.3 per cent mercuric chloride gave 94 per cent germinations.

Seed wheat treated 10 minutes or less with 1 to 2 per cent solution of formalin gave a normal number of germinations or better, but soaking over 10 minutes decreased slightly the per cent of germinating seeds. Exposure for 10 minutes to 10 per cent killed all, and merely dipping into 5 per cent reduced the germinations to 34 per cent. Subsequent experiments showed that wheat or oats would germinate perfectly after soaking in 0.4 per cent formalin 1 to 3 hours.

Seed wheat will stand an exposure of 1 minute at 150° F. (65.5° C.) and give 80 to 90 per cent of germinations. Oats exposed to hot water at 140° to 143° F. for 5 minutes gave

98 per cent germinations and exposure for 5 minutes in water at 140° F. (60° C.) or below may be recommended as not injurious to wheat.

Barley dipped for 30 minutes in copper sulphate water (1 pound to 4 gallons) gave 86 per cent of weak germinations; and when exposed for 1 hour, 47 per cent.

Wheat exposed to potassium sulphide (1 ounce to 1 gallon for 75 minutes gave 100 per cent germinations. Barley treated in the same way for 75 minutes gave 90 per cent germinations. Oats treated in same way gave 96 per cent germinations; but exposed for 19 hours gave 42 per cent weak germinations.

According to Crancfield (1901), formalin used as weak as 2.5: 1,000 (1 pound [pint] to 50 gallons of water) for 20 minutes may injure oats used for seed. The experiments cover 20 varieties of oats, and the germination of over 25,000 seeds. The amount of injury varied greatly in different varieties, and was more noticeable in planted seeds than in those used in the germinating chamber.

Longer exposures than 20 minutes at the standard strength (1 pint to 50 gallons) did not much increase the injury. The early growth from the treated seed was retarded and at no time did the treated quite equal the untreated in height.

When more concentrated solutions of formalin were used the injury was progressively greater, *e. g.*, 1 pint to 50 gallons of water, 91 per cent germination (check 94.5); 1 pint to 25 gallons of water, 74 per cent; 1 pint to 20 gallons, 73 per cent; 1 pint to 10 gallons, 31 per cent; 1 pint to 5 gallons, 12 per cent.

In 1901, Windisch published many experiments on lupins, peas, horse beans, soy beans, corn, flax, rape, lucern, and clover, showing the effect of formaldehyde on germination. Each of these was in duplicate. The following are some of his conclusions:

*Per cent of Germinations.*

	No. of seeds each.	Distilled water.	Formaldehyd (Per cent in Water.)				
			0.02	0.05	0.10	0.20	0.40
White lupins.....	50	100	100	99	89	4	4
Victoria peas.....	50	84	80	56	19	6	12
Horse beans.....	50	100	97	100	98	94	26
Soy beans.....	100	99	98	97	92	40	6
Flax.....	200	97.75	94.25	11.75	.....	.....	.....
Maize.....	100	100	100	.....	99.5	100	94
Summer rape.....	200	98.25	80.25	4	2	.....	.....
Lucerne.....	200	90.50	88.75	27	7.75	7	7.5
Clover.....	200	95.00	89.50	34	7.5	8	4.5

No injurious action was observed on lupins, peas, horse beans, soy beans or maize, when the 0.02 per cent formaldehyde solution was used.

Hiltner, in some root-nodule experiments, exposed soy bean seeds for 3 minutes and for 10 minutes to 1:100 mercuric chloride water, then carefully washed it away and planted. The plants came up badly, but this was not ascribed to the germicide.

According to Dr. Windisch, winter wheat endured a soaking for 24 hours in 0.02 per cent formaldehyde, and in 0.04 per cent without lessening the power of germination. It also endured 0.08 per cent formaldehyde for 24 hours and gave a germination of 88.5 per cent at the end of 14 days. Exposure to 0.12, however, gave only 9.25 per cent germination at the end of 14 days, and exposure to 2 per cent gave 0 per cent germination at the end of 14 days. Even the diluted solutions delayed the germination somewhat.

F. L. Stevens (1909) reports that treatment of oats with a solution of 1 ounce formalin to 5 gallon of water reduced germination to 37 per cent, while a solution of 1 ounce to 1 gallon of water for 24 hours gave a germination of 73 per cent to 96 per cent, according to

the varieties used. He recommends for practical purposes a solution of 1 ounce to 1 gallon for 2 hours, followed by 10 hours treatment with lime.

#### GERMICIDAL TREATMENT OF DORMANT PLANTS.

Plants in the resting condition, especially roots and shoots protected by cork, will bear relatively strong doses of germicides. Bordeaux mixture (6:4:50), copper sulphate solution (3:50), mercuric chloride water (1:1000), soap and lye solutions, lime washes, cold boiled or hot boiled lime sulphur solution, lime-salt-sulphur wash may be applied rather freely. A few formulæ are given at the end.

A more difficult and important problem concerns the use of germicides and insecticides on growing plants.

#### GERMICIDAL TREATMENT OF GROWING PLANTS.

In the treatment of growing plants two things must be kept in mind constantly:

(1) The foliage must not be injured; (2) the applications must be effective. A third very desirable quality in a germicide is adhesiveness, since if the substance is washed off by every rain the necessary reapplications will be expensive.

Bordeaux mixture containing an excess of lime, *e.g.*, formula 4:6:50, or 4:4:50 is borne very well by some plants. The foliage of others is liable to be burned, especially if the spraying is not repeated frequently either with Bordeaux or with milk of lime so as to keep an excess of lime present on the leaves. This mixture is an effective fungicide and also has some value as a germicide. Pierce used it on walnut blight in California with partial success. It might perhaps be used to protect from some of the leaf spots. Always, however, it is advisable to try the experiment on a small scale first, until the general effect of the copper on the foliage has been determined. The writer has seen a whole peach orchard defoliated in midsummer by the improper use of Bordeaux mixture.

Copper absorbed in minute quantities, has a stimulating effect on growth. Chester observed this in 1890 while testing the effect of copper salts on *Vitis*. He says that Bordeaux mixture seems to stimulate the growth of the vines.

In 1894, Frank & Krüger stated that the assimilation of potato leaves is increased, the transpiration becomes greater, the leaves live longer, the harvest is increased, and the tubers contain more starch when the plant has been treated with copper salts, especially "the ordinary 2 per cent copper vitriol-lime mixture."

In 1895 Galloway and Woods showed that Bordeaux mixture could be used with safety on growing grape-vines and potatoes, and observed that copper salts stimulated the growth of these plants.

In 1898, Harrison stated that Bordeaux mixture has an invigorating effect on the foliage of plum, pear, peach, and quince.

In 1898, Starnes in Georgia reported injury to peach foliage from copper salts sprayed thereon.

In 1899, Duggar obtained shot-hole effects on peach foliage as the result of the use of copper fungicides.

In 1900, Pierce published his observations on the physiological stimulation of Bordeaux mixture on peach leaves.

As a result of his researches, published in 1902, Bain concludes that peach leaves are especially sensitive to poisons in general and to copper in particular.

The self-boiled lime-sulphur mixture is less injurious to the leaves of peach and plum trees than Bordeaux mixture, and appears to be an equally good germicide. Scott has used it on peaches for the prevention of the leaf-spot due to *Bact. pruni*, and with brilliant success in the summers of 1909 and 1910 for prevention of the brown rot due to *Monilia*. It should be tried also for the prevention of the walnut blight due to *Bact. juglandis*.

The stage at which cold water should be poured on to stop the cooking varies with different limes. Some limes are so sluggish in slaking that it is difficult to obtain enough heat from them to cook the mixture at all; while other limes become intensely hot on slaking and care must be taken not to allow the boiling to proceed too far. If the mixture is allowed to remain hot fifteen or twenty minutes after the slaking is completed, the sulphur gradually goes into solution, combining with the lime to form sulphides which are injurious to peach foliage. It is therefore very important, especially with hot lime, to cool the mixture quickly by adding a few buckets of water as soon as the lumps of lime have slaked down. The intense heat, violent boiling and constant stirring result in a uniform mixture of finely divided sulphur and lime with only a very small per cent of the sulphur in solution. The mixture should be strained to take out the coarse particles of lime, but the sulphur should be carefully worked through the strainer. (From Scott and Ayres' Bulletin on The Control of Peach Brown-Rot and Scab.)

Alsberg and Hasselbring in U. S. Department of Agriculture in the summer of 1909 subjected cabbage leaves to 1:200 mercuric chloride water for 30 minutes without entirely sterilizing their surfaces, *i.e.*, a white schizomycete subsequently appeared in the flasks containing the leaves which were to have been examined chemically. In this instance, however, the leaves were rather large and were not previously soaked in alcohol.

### INSECTICIDES.

Carbon bisulphide is an excellent insecticide for certain purposes. Its vapor is inflammable and care should be exercised in its use. It must not be used near an open flame.

In 1897, Hicks and Dabney showed that there was no appreciable loss of germinating power in wheat, corn, barley, or rye, from treating the seed in bulk with carbon bisulphide for 24 hours at the rate of one pound of the chemical to 100 bushels of grain.

In recent years it has come to be recognized that carbon bisulphide is better adapted to kill certain insects, *e.g.*, weevils in grain, phylloxera in the soil, etc., than hydrocyanic acid gas, because it has greater penetrating power. One teaspoonful per cubic foot is the usual amount allowed in making small treatments (Jno. B. Smith).

For aphides on plants in the open air, kerosene emulsion is useful. To make it one must have a force pump with good churning power. When properly made it may be kept for some weeks and diluted with water as needed for spraying.

For aphides in houses tobacco smoke properly applied is very effective and not injurious to the plants. Improperly applied, it may burn the foliage seriously. The houses should be well wet down in advance, and then a prepared tobacco paper burned until there is a dense smudge, or else the house filled with the steam from strong tobacco water. This latter may be obtained by distributing shallow pans of the concentrated fluid at frequent intervals and dropping large red hot spikes into the liquid from a wire crate which has been heated in the engine-room furnace. It may also be evaporated from pans placed over oil stoves. This concentrated tobacco extract may be had on the market under the name of Nicofume.

Aphides and most other pests in houses may be destroyed by hydrocyanic acid gas. This treatment is inexpensive and very effective. Only red spiders do not seem to be much harmed by it at least in such doses as can be used on plants. Plants are also sensitive to this poison but to a less degree than most animals. Different varieties of plants also vary considerably in sensitiveness. The tomato and olive are quite sensitive. The aim of the grower should be to generate just enough of the gas per cubic foot to destroy the insects without injuring the plants. If the grower has no knowledge of the amount of gas which his crop will tolerate, then he must determine this on a small scale before applying the remedy to a whole house, otherwise disastrous results are likely to follow.

Eggs of insects, *e. g.*, those of the white fly (*Aleyrodes*), are more resistant to this gas than the mature forms, or the plants infested, and therefore small doses of the gas at

frequent intervals as the eggs hatch are necessary to control certain pests, *i. e.*, as often as twice a week for a month, if the houses are badly infested.

The gas is best generated in stone jars which should be distributed at equal distances through the house, and not set too close to the plants lest the near ones should receive an overdose of the gas and be scorched.

The jars are dosed with a measured amount of crude sulphuric acid and water (1 to 2), and into these are dropped weighed amounts of cyanide of potash wrapped in thin brown paper so as to delay the evolution of the gas for a minute, and thus allow the operator to escape. To avoid the boiling over of the acid during the violent evolution of the gas, the jars should be deep rather than shallow. The house should be shut tight and arrangements made in advance to open it from the outside when the exposure is completed. The cyanide of potash may also be lowered into the jars from the outside by means of strings; this is a rather safer way since the generated gas diffuses through the air with great rapidity, *i. e.*, nearly as fast as a man can run. Sunset of a still day is the best time for commencing the exposure. The house should be opened up after 1, 2, or 3 hours.

The air space of each house must be calculated very carefully and for growing plants not more than 0.15 gram of the cyanide of potash should be used for each cubic foot and half this quantity for sensitive varieties. Any serious error in the calculation means, of course, either failure of the treatment or destruction of the crop. The gas is deadly to man and the higher animals, and exposures must not be made in lothouses connected with stables or living rooms; and if dwelling houses are near, the doors and windows on that side must be closed, or the rooms vacated. The potash salt is also very poisonous and must be kept out of the reach of children and animals and handled with rubber gloves.

A good remedy for red spider is a desideratum. Repeated syringing with water is recommended. They are usually worse in dry seasons.

For the destruction of larvæ, beetles, and bugs out of doors, a spray containing arsenate of lead is effective, and foliage usually bears this poison very well, *i. e.*, much better than Paris green. Popenoe used 6 pounds to 50 gallons of water on potato foliage to destroy the Colorado potato beetle. All the larvæ were killed in 48 hours and the plants were not injured. Paris green is also an effective insecticide. It may be sprayed on the foliage, which is sometimes burned; or may be dusted on mixed with flour, air slaked lime, or land plaster (1 part to 30 or 50).

Both lead arsenate and Paris green may be combined with Bordeaux mixture, so as to avoid two separate sprayings.

For suggestions respecting trap crops see pp. 282, 295, 296.

Many of our experiment stations now publish annual spraying calendars and other literature, giving the principal formulæ, and usually these publications may be had upon application.

## FORMULÆ.

*Copper Sulphate.*

Dissolve in hot water, or by suspending the crystals in a sack in the top of the cold water. This is best done over night. It is convenient to make a strong solution (1 or 2 pounds per gallon) and dilute as needed.

*Ammoniacal Solution of Copper Carbonate.*

Use 5 ounces of copper carbonate, 3 pints or less of strong ammonia water (26° B.), *i. e.*, just enough to bring the copper carbonate into solution, and 50 gallons of water. The copper carbonate must be wet with water first and then stirred into the ammonia after the latter has been diluted with 5 or 6 volumes of water. Add always a slight excess of the copper carbonate and use only the supernatant clear liquid.

Used on foliage and fruit when Bordeaux mixture would be unsightly. Apply frequently in case of rainy weather.

*Bordeaux Mixture.*

There are various formulæ in which the first figure represents pounds of copper sulphate, the second figure pounds of stone lime and the third gallons of water. The 6 : 4 : 50 is the usual combination and sticks better than the 4 : 6 : 50. It also sprays easier. For sensitive plants 4 : 4 : 50 may be used or 4 : 5 : 50.

The lime must be of good quality and fresh slaked. Air slaked lime must not be used, neither should concentrated solutions be mixed, nor hot solutions. Divide the 50 gallons of water into two equal parts. Dissolve the copper sulphate in one part. This is usually done over night. Slake the lime with a portion of the other part, adding the water slowly, then add the remainder of the water when the lime has ceased to be lumpy. When ready to spray, stir thoroughly to obtain an even mixture of the lime and water and pour the two fluids together through a strainer tied across the top of a clean barrel. The two streams should blend as they fall to insure a good product, the essential features of which are alkalinity and a fine grain insuring suspension in the fluid long enough to permit of the spraying, which should be undertaken at once. Concentrated solutions give a coarse precipitate which settles quickly. The mixture should be absolutely free from sawdust, sticks, straws, chaff, wool, fragments of leaves or any similar substances. Otherwise, vexations delays are likely to arise from clogging of the nozzle. In spraying use

a good force pump. A Vermorel nozzle affords a well distributed fine spray. For dilute Bordeaux reduce the copper sulphate one-half, or double the volume of water. This may be sprayed upon soils to check the damping off of seedlings.

Stock solutions of the two fluids may be prepared in advance and will keep indefinitely. They are conveniently kept in tubs or half barrels closely covered, the lime always under the required volume of water, and the copper sulphate in strong solution. When needed one then has only to measure out a portion of the copper sulphate water, dilute it to the required volume, stir up the settled lime very thoroughly, dip out the required volume of the milk of lime quickly, and pour the two fluids together, as already described.

An acid Bordeaux should never be sprayed upon plants. The following are tests for acid Bordeaux: (1) A film of metallic copper deposited on polished iron or steel when plunged into the mixture; (2) a purplish red reaction on putting into the Bordeaux a drop of a water solution of yellow prussiate of potash (1 to 10). If either of these reactions is obtained more lime must be added. It is best to avoid *dry* Bordeaux and similar commercial substitutes.

*Resin Bordeaux.*

This is made by adding to each 50 gallons of Bordeaux a clear liquid made by boiling for one hour 1 pound resin and 0.5 pounds crystals sal soda in 0.5 gallon water. Another way of making it is to melt 5 pounds of resin in 1 pint of fish oil, slowly add 1 pound of potash lye, stirring, and taking care that it does not become too hot and boil over. Then add 2 gallons of water and continue boiling for an hour. Finally add slowly with stirring an additional 3 gallons of water. The finished product should dissolve readily in cold water. Two gallons of this soap is added to each 50 gallons of the finished Bordeaux. Resin fish oil soap may also be bought and may be added to Bordeaux at the rate of 5 pounds per 50 gallons.

*Soda Bordeaux.*

Soda lye 1 pound; copper sulphate 3 pounds; lime 5 ounces; water 50 gallons.

*Arsenate of Lead.*

Three pounds to 50 gallons of water, mixed thoroughly with a little water first.



*Arsenite of Lime.*

Arsenic, 1 pound; stone lime, 4 pounds; water, 4 gallons. Boil half an hour then dilute to 200 gallons with water.

*Paris Green.*

Paris green, 0.5 pound; lime, 1.5 pounds; water, 50 gallons, or perhaps better: Paris green, 1 pound; quick lime, 3 pounds; water, 250 gallons.

*Combined Bordeaux Mixture and Paris Green or Arsenate of Lead.*

Add to the regular 50 gallon Bordeaux, 1 pound of Paris green stirred up thoroughly in a gallon of water, and stir thoroughly afterwards. If arsenate of lead is used, double the amount may be added in the same way.

*Boiled Lime-Sulphur.*

Lime, 25 pounds; sulphur, 17.5 pounds; water, 50 gallons. Boil 1 hour. Apply at once.

*Self-Boiled Lime-Sulphur (Scott's method).*

Sulphur, 10 pounds; stone lime, 15 pounds; water, 50 gallons. Put the lime into a barrel and pour over it 2 to 3 gallons of boiling hot water, add the sulphur at once, then 2 or 3 additional gallons of the hot water. Stir frequently. More water may be added if it becomes too thick, but add as little as possible. The cooking should take place in about 6 to 8 gallons of water. The mouth of the barrel should be covered to retain the heat. When slaked add the remainder of the water, *i. e.*, cool quickly. Strain. Apply at once.

*Lime-Salt-Sulphur.*

Best stone lime, 30 pounds; sulphur, 15 pounds; salt, 10 pounds; water, 50 gallons. Slake the lime in hot water, then while hot add the sulphur and enough water to make a thin paste and boil for three-fourths hour, stirring thoroughly, adding more water as it evaporates. Then add the salt, boil an additional 15 minutes, dilute with hot water, filter and spray hot.

Warren gives the following method of preparation: Fresh lime, 15 pounds; flowers of sulphur, 15 pounds; salt, 15 pounds; water, 45 gallons. Bring 4 or 5 gallons of water to a boil in an iron kettle, mix the sulphur with hot water, crushing the lumps, then put into the boiler, add the lime in 4 separate parts, adding cold water gradually to subdue the violent boiling and prevent from overflowing. Finally add the salt, boil 1 hour or more, stirring frequently. Strain, dilute with the remainder of the 45 gallons (about two-thirds) and spray.

There are other formulæ in which the proportions vary somewhat.

*Potassium Sulphide.*

One pound to 50 gallons of water. To be used at once, because it soon loses strength.

*Carbon Bisulphide.*

Use 1 pound to each 100 bushels of grain, or 1 teaspoonful to each cubic foot of space.

*Mercuric Chloride.*

Solution of 1 part to 1000 parts of water. To be used in glass or wooden vessels, never in metal ones. For field use tablets may now be purchased so that it is only necessary to dissolve the requisite number in a given volume of water.

Seed corn may be exposed 20 minutes with entire safety, wetting first in alcohol for a minute or two. Unsprouted potatoes 40 minutes to 1 hour.

*Hydrocyanic Acid Gas.*

For treating dormant nursery stock, W. E. Britton, recommends 1 ounce cyanide of potash, 2 ounces sulphuric acid and 4 ounces of water for each 100 cubic feet of space. The acid is poured into the water, never the reverse, on account of over heating and danger of steam explosions; the cyanide is added, and the room shut up tight for half an hour. For greenhouse fumigation Woods and Dorsett recommended 20 minutes' exposure using 0.075 gram to 0.15 gram cyanide of potash per cubic foot, depending on the kind of plants, ferns being very sensitive, and violets rather resistant. Symons has shown that dormant peach buds will endure 0.50 gram of potassium cyanide per cubic foot (2 ounces per 100 cubic feet) for 60 minutes. Apples will endure as much. The use of 0.30 gram per cubic foot for 30 minutes is scarcely sufficient to kill all of the San José scale, but 0.30 gram for 45 minutes would be.

*Formalin (40 per cent Formaldehyde).*

1 pint to 50 gallons of water for smut of wheat and oats;  
2 pints to 50 gallons for scab of potato;  
4 pints to 50 gallons for disinfection of soils.  
The formalin should be taken from sealed (fresh) bottles, as it loses strength readily.

*Hydrogen Peroxide.*

Use 1 part to 200 of water. Must be fresh

*Whale Oil Soap.*

This may be used for plant lice at the rate of 2 pounds per 12 gallons of water. Dissolve in hot water. In greater concentration it should be tried in advance on a few plants. In proportion of 1 pound to 4 gallons of water it is said to injure tender plants (J. B. Smith).

*Tobacco with Whale Oil Soap.*

Boil 3 pounds of dry tobacco stems or leaves in 10 gallons of water and add while hot 0.5 pound whale oil soap.

*Soap.*

Use 1 pound to 8 gallons of water.

*Kerosene Emulsion.*

Dissolve 1 pound of soap in 2 gallons of hot water, add 4 gallons of kerosene and churn for 15 minutes with a force pump. The thick creamy emulsion, which does not separate

readily, is diluted for use with 9 parts of water and sprayed at once. Use rain water. If a good emulsion has not been obtained, do over. An imperfect emulsion must never be sprayed.

*Soluble Oil.*

This substance, sold under various names as Kill-o-Scale, Target Brand Scale Destroyer, is sprayed on the dormant plants after proper dilution (1 : 20).

*Formalin Vapor.*

For freeing closed spaces from bacteria Schering's formalin lamp may be used.

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# VASCULAR DISEASES.

## WILT OF CUCURBITS.

(*Synonyms:* Cucumber-wilt; Cantaloupe-wilt; Squash-blight; Pumpkin-blight).

### DEFINITION.

This is a specific communicable disease of cucumbers, squashes, and some allied plants. It is characterized by the sudden wilting and shriveling of the foliage and by the presence in the vascular bundles of enormous numbers of a white sticky bacillus which is the cause of the disease.

### HOST-PLANTS.

This disease has been observed in cucumbers (*Cucumis sativus*), muskmelons (*Cucumis melo*), pumpkins (*Cucurbita pepo*), and squashes (*Cucurbita moschata* and *C. maxima*). The disease has been successfully inoculated by the writer into all of the above mentioned plants many times over and into the following additional cucurbits: *Cucumis odoratissimus*, *Benincasa cerifera*, *Cucumis anguria*, *Cucurbita foetidissima*, *C. californica*, *Sicyos angulatus* and *Echinocystis lobata*, the last four being wild plants of the United States. The disease is not known to the writer to occur in the watermelon but it has been reported by Selby. Inoculations into this plant, while sometimes producing a wilt of the pricked leaf generally failed to induce any secondary wilt. In one or two instances other leaves than the inoculated ones wilted and the vessels were found plugged by a bacillus. Often there was no wilt even in the punctured leaves. Inoculations from virulent cultures into the following cucurbits failed, or produced only local injuries from which the plants recovered: *Melothria scabra*, *Cucumis erinaceus*, *Luffa acutangula*, *Momordica balsamina*, *Lagenaria vulgaris*, *Trichosanthes cucumeroides*, *Apodanthera undulata*. The disease is not known to occur in any wild plant, but it is so very easily inoculated into *Sicyos angulatus* and *Cucurbita foetidissima* that it should be searched for on these plants and on other wild cucurbits.

Inoculations into non-cucurbitaceous plants such as *Solanum tuberosum*, *Lycopersicon esculentum*, *Datura stramonium*, *Passiflora incarnata*, *Vigna catjang*, *Nicotiana tabacum*, *Pyrus orientalis* and *Hyacinthus orientalis* yielded only negative results. The disease is not known to occur outside the Cucurbitaceae, and probably many species of plants within the limits of this family are not subject to it.

### GEOGRAPHICAL DISTRIBUTION.

The limits of this disease are not known. It occurs in Canada, Massachusetts, Vermont (?), Connecticut, New York, New Jersey, Delaware, Maryland, Virginia, West Virginia, Pennsylvania, Kentucky, Ohio, Indiana, Illinois, Michigan, Wisconsin, Missouri, Iowa, Nebraska and Colorado. It is not known to occur south of latitude 35° and it is believed to be restricted in its southern distribution by the fact that the bacillus is very sensitive to heat. It should be searched for, however, in the Gulf States. The disease has been reported from Germany by Dr. Otto Appel; from Russia, near St. Petersburg, by Dr. Iwanoff; and it is to be looked for in all north-temperate regions where cucurbitaceous plants are grown and where the temperature to which the vines are exposed does not exceed the thermal death-point, or the maximum temperature for the growth of this organism.

Nothing is known to the writer concerning its occurrence in the far East or in the south-temperate zone except a statement to him in 1910 by I. B. Pole Evans that it occurs on pumpkins in the Transvaal.

Contrary to certain statements the disease occurs in hothouses as well as in the fields, but it is more generally prevalent in open-air culture than under glass. It occurred naturally, however, two different years in a hothouse near Washington and in 1899 the writer identified it from a hothouse at Morrison, Illinois, where it did considerable injury. The organism was cultivated out of plants from both houses and the disease was reproduced by inoculations from these cultures.

It is perhaps worth while to discuss the occurrence of this disease in the United States at greater length. This disease was first observed by the writer (in 1893) near Washington, D. C. (plate 13), and has been observed in fields and gardens around Washington every year since 1893 (fig. 50).

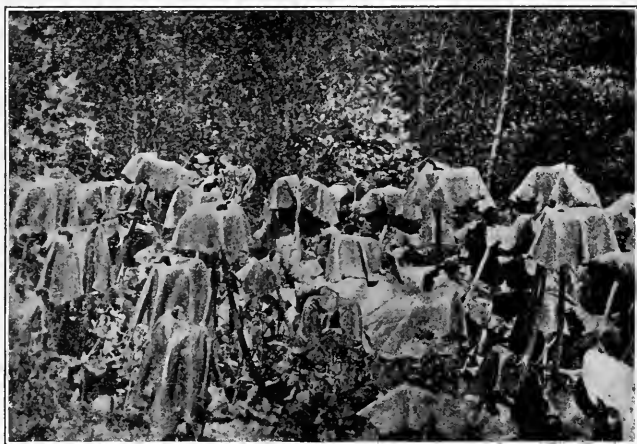


Fig. 50.\*

I saw it at Chuckatuck in Nansemond Co., Virginia, in 1898. This is the farthest south I have seen it.

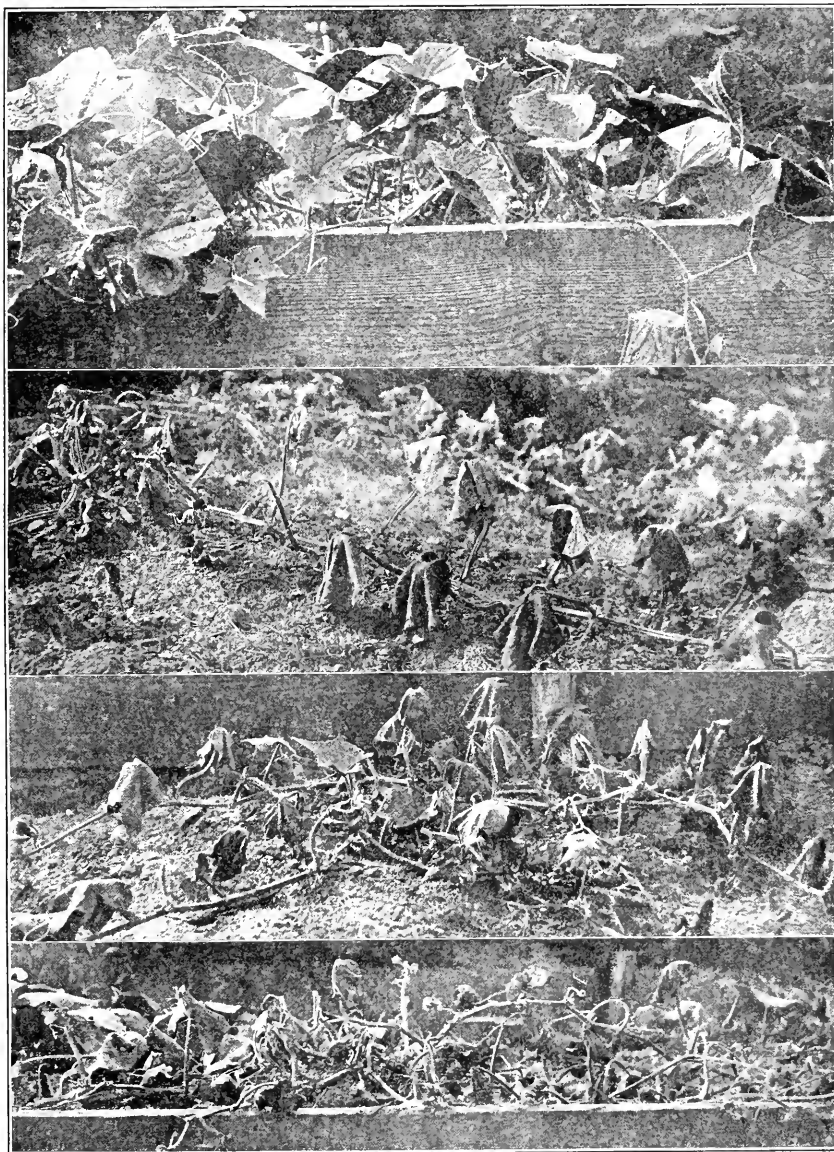
It was cultivated pure from the interior of cucumber-stems received from Bristow, Prince William Co., Virginia, July 19, 1897.

Nothing is known of its occurrence south of Virginia. It might be looked for farther south in the mountains, but hardly on the flat lands of the far South owing to its low thermal death point. It is northern rather than southern in its distribution.

It occurs in Pennsylvania and throughout New Jersey and Delaware, at least in places. In September 1901, I saw the disease in cucumbers at Woods Hole, Mass., and in October 1903, in muskmelons farther north on Buzzard's Bay. I received it from Connecticut in 1905 (melons), and from Rhode Island in 1911 (cucumbers). It probably occurs all over New England.

I saw it in a field of cucumbers on the western end of Long Island in July, 1902, at least one-third of the vines being affected. Gnawings due to the striped beetle (*Diabrotica*) were numerous and many of the wilted spots originated from the bitten places (see Etiology).

\*FIG. 50.—Patch of diseased Hubbard squashes on place of David Fairchild, at Chevy Chase, Md. Plants wilted by *Bacillus tracheiphilus*. Summer of 1907. Photographed by Mr. Fairchild.



Cucumbers showing various stages of bacterial wilt due to *Bacillus tracheophilus*.

Photographed by writer July 13, 1893, on a hill-side at Anacostia, D. C. Fig. 1 (top), healthy vines for comparison with diseased plants on same terrace. Fig. 2, primary and secondary wilt. Leaf blades toward base of stem (left) have been destroyed by direct infection (beetles) and bacteria have penetrated into main axis. Leaves toward apex of stem have wilted as result of disturbed water-supply due principally to occlusion and destruction of vascular bundles in stem. Fig. 3, only two third leaves remain; petioles and stems were still green and normal in appearance. Bacteria filled the spiral vessels of main shoots with a white viscid slime. Fig. 4 (bottom), no sound leaves; stems beginning to shrivel.





Some years ago near Albany, New York, it did much damage to fields of cucumbers according to John E. W. Tracy of the U. S. Department of Agriculture, who also saw it near Rochester.

This disease was common in squashes and cucumbers at Hubbardston, Michigan, in 1895 and subsequently.

On August 20, 1897, I saw near Saginaw, Michigan, two fields of cucumbers in which the disease was present. There were vines in all stages of wilt from those just beginning to be affected to those which were dried up. When I made cross-sections of the stems toward the root and touched the cut end with my finger the bacilli strung out in the characteristic way in delicate sticky threads. On showing the plants to a cucumber-grower he said he was familiar with the disease but did not know its cause.

During the same month I saw this wilt in cucumbers and squashes at Grand Rapids, Michigan.

In 1898, Mr. S. S. Bailey, of Grand Rapids, Michigan, lost many of his squashes by this disease.

It was seen by the writer in muskmelons at Racine, Wisconsin, in 1897. It has been reported by Prof. Pammel from Iowa.

Mr. Ragan, the horticulturist tells me he has seen it in Indiana.



Fig. 51.\*

Specimens of cucumber attacked by this disease were received by me from Morrison, Ill., in 1899. The slime was so sticky that when touched with the finger, it strung out from the end of the cut stem over 76 cm. (30.5 inches). It also occurs in the vicinity of St. Joseph, Mo.

Mr. Barlow has observed it at Guelph, Ontario, in a variety of cucurbits.

#### SIGNS OF THE DISEASE.

This disease is readily detected owing to the striking nature of the phenomena. The wilt is first local, affecting certain individual leaves (plate 1, fig. 1 and various text figures), but soon becomes general, involving the foliage of the entire plant (plates 13 and 14). Associated with the wilt we always find a white ooze exuding from the vascular bundles of leaves or stems on cross-section (fig. 51), and this exudate is usually viscid. The only other diseases of cucurbits liable to be confounded with this are: (1) The more or less sudden wilt due to the presence of the larvæ of the squash-vine-borer (*Aegeria cucurbitae*) in the base

\*FIG. 51.—Cross-sections of cucumber-stems, showing bacterial ooze (*Bacillus tracheiphilus*) from the bundles. Plants from New York. Photographed Aug. 11, 1904. Enlarged about ten times.

of the stem or main root; (2) A sudden wilt due to the filling of the vascular bundles with fungi of the form-genus *Fusarium*; and (3) A wilt due to the rotting off of the main stem at the surface of the earth. This disease may be distinguished readily enough by the facts that fungi are not present and that there is no stem-injury or root-injury of the kinds just described, and also by the further fact of the invariable presence of large numbers of white, sticky bacteria in the vascular system. These are so abundant and usually so viscid that if the tip of the finger be pressed against the cross-section of a diseased stem at once, or better, some minutes after cutting, and then gently removed, the bacteria will remain attached to the finger and string out in numerous delicate threads (fig. 52) resembling cobwebs. For the microscopic structure of these threads consult Vol. I (fig. 14). If a little time is allowed the bacteria also ooze from the cut surface (cross-section) of such stems in milk-white drops, especially if the stems are cut a second time and the basal end put into water or moist air. The wilting and shriveling of the leaf blades always precede the destruction of the leaf-stalks and of the stem by a considerable period, so that it is common to find plants which have lost all or nearly all of their foliage while still retaining a green and normal looking stem (plate 1, fig. 2), the vessels of which, however, for long distances will be found to be



Fig. 52.\*

more or less fully occupied by the bacillus (fig. 6). In the end, petioles and stems shrivel and die, but the organism does not make its appearance on the surface of the plants and there is nothing resembling a soft wet-rot, not even in the fruits. In rather resistant plants, e. g., certain squashes, the foliage may wilt during warm, dry days and partially recover at night or during cool, moist days, to wilt again when the demands of transpiration are greater. In such plants there is dwarfing (fig. 53) accompanied, in some instances at least, by an excessive blossoming and branching. In the cucumber and muskmelon the disease is, on the contrary, quite speedily destructive, a few weeks after the close of the period of incubation being generally sufficient to destroy the plants. There is in these species much less tendency to recover from the wilt temporarily during cool or wet weather than there is in the squash, and the writer has not observed any proliferation of shoots or flowers. When the disease is active there is seldom any yellowing of the foliage in advance of the wilt. The loss of turgor and change of color (from bright green to dull green) are sudden. The characteristic signs are well exhibited

in the accompanying illustrations.

The disease generally starts in the center of a hill, i. e., on the blades of the basal leaves which soon shrivel. In this stage of the disease in that part of the main axis near the primary infections the vascular bundles, and especially the spiral vessels, are gorged with the bacteria and there are usually many bacterial cavities in the primary vessel-parenchyma (fig. 54).

#### ETIOLOGY.

The cause of this disease is a white peritrichate schizomycete named by the writer *Bacillus tracheiphilus* from its special fondness for the vessels of the plant. This organism was first isolated and described by the writer (1893-95), and most of the statements here given rest upon his own observations and experiments, which now cover a period of 18 years. The disease is very readily induced by needle-punctures without hypodermic injection. All that is necessary to produce the disease is to dip the end of a sterile steel needle into a recent

\*FIG. 52.—Viscid threads of *B. tracheiphilus* stringing from cut end of a cucumber-stem. Plant from field, autumn of 1904. Threads slightly diagrammatic, i. e., not sufficiently cobwebby.



#### Wilt of Cucurbits.

Cucumber-plants inoculated with *B. tracheiphilus* in summer of 1903 (hothouse, U. S. Department of Agriculture, by James Birch Rorer).

Infections were by means of needle-pricks, using pure cultures. In each case needle-pricks were confined to a single leaf-blade. This blade first wilted and then gradually all the other leaves. Inoculations were made on July 23 and photograph about 18 days later. Much reduced.



pure culture on steamed potato or nutrient agar, or in beef-bouillon, and make a few delicate punctures into a susceptible plant, *e. g.*, into the blade of a cucumber-leaf or muskmelon-leaf. The period of incubation in the writer's experiments has varied from 3 to 31 days and must depend partly at least on the number of bacilli inserted. Ordinarily when 20 or 30 needle-pricks are made the first signs appear in from 5 to 9 days in the punctured part of the leaf (figs. 56, 60, 63, 74). When young cultures are used on very susceptible plants such as *Cucumis sativus*, *Cucumis melo*, or *Cucurbita foetidissima*, the disease appears with the certainty and regularity of clock-work. It is more difficult to inoculate squashes successfully, at least with some strains of the organism, and this corresponds to the observed fact that they are more resistant in the field. One winter, on several kinds of squashes the writer experienced repeated failures, using virulent cultures obtained from the cucumber. The pricked cucumber-plants and muskmelon-plants contracted the disease; the squashes, both summer and winter varieties, inoculated at the same time, in the same way, and from the same cultures, resisted, or only showed traces of primary wilt. This

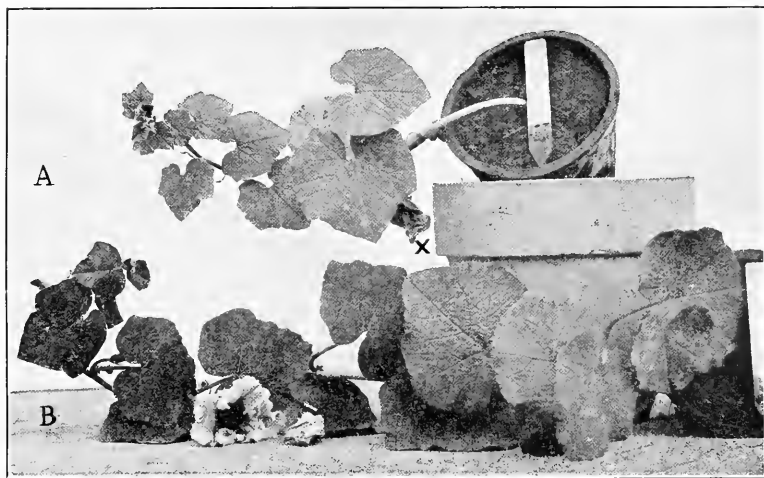


Fig. 53.\*

resistance may be due to some extent to varying degrees of virulence on the part of particular strains of the organism; or to varying degrees of resistance on the part of the host. Possibly the squash bacillus should be regarded as a variety.

In the summer of 1905 the question of the identity of the squash-wilt and cucumber-wilt was gone over once more. Inoculations made into four varieties of squashes, using a strain isolated the previous year from a muskmelon, and proved by numerous control-experiments to be virulent to cucumbers, would not infect squashes. A little later in the season the same squashes were readily infected with a strain of the bacillus isolated from the vessels of a wilting squash-plant found in a garden in Washington, and the same cultures

\*FIG. 53.—A. Winter squash, variety Pikes Peak, No. 215, inoculated Oct. 5, 1895, by needle-pricks on two leaf blades, using viscid white slime from a cucumber-stem. Both leaves contracted the disease and shriveled slowly, one of them being shown at X. Although the plant was under observation for 66 days the only additional signs of disease were conspicuous dwarfing with yellowing of the foliage, especially the lower leaves. Photograph made Dec. 10; plant then cut and examined under microscope, bacteria being demonstrated in a few vessels of several (5) bundles. B. An uninoculated plant from the same lot of seedlings. About one-sixth natural size.

proved equally virulent to cucumbers, the disease occurring promptly and the signs being typical in all respects, including the presence of the sticky bacillus in the vascular system (plate 15, fig. 1). The inoculation-experiments were repeated a few weeks later with the same positive results; squashes and cucumbers being infected with uniform success. Additional studies should be made.

In the inoculated plant, the primary foliar signs (a dulled green with absence of turgor) always appear first in the punctured area and immediately around it, but never until after a definite period of incubation covering at least several days. The signs of disease gradually extend until the entire blade of the leaf is involved. The loss of turgor and change to dull green is soon followed by shriveling, after which the leaf-blade becomes brown. Subsequently, and usually considerably prior to the collapse of the petiole of this leaf, the blades of other leaves up and down the stem suddenly wilt (plate 16 and text fig. 58). The first leaves to show this secondary wilting are ordinarily those which arise from parts of the stem nearest to the insertion of the inoculated leaf; exceptionally the first leaf to show secondary wilt is one standing over the inoculated leaf rather than one actually nearer but inserted on the opposite side of the stem. Gradually more and more remote leaves are

destroyed until the whole plant is involved. Whenever this secondary stage of the disease supervenes, the vessels in the stem (which still outwardly presents a green and normal appearance) will be found to be occupied more or less fully by the bacillus. Usually the organism is to be found in the vessels of such plants in extraordinarily large numbers. In the stem of the squash the writer traced the bacterial occupation microscopically in one plant to a distance of 210 cm. from the point of infection, and in another plant to a distance of 240 cm.

Almost all of the writer's inoculations have been made by means of needle-punctures into the blade of the leaf, at first often directly from plant to plant, but in recent years generally from pure cultures (descendants of poured-plate colonies) on agar, carrot, potato or in beef-bouillon, and other fluids. This method closely resembles

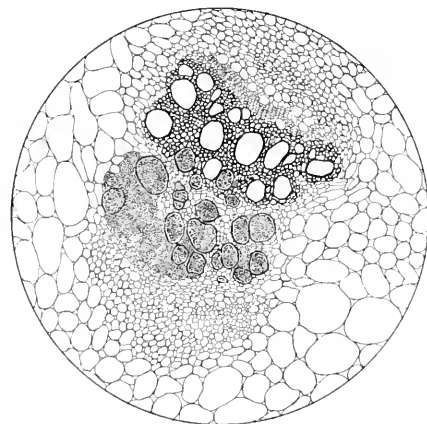
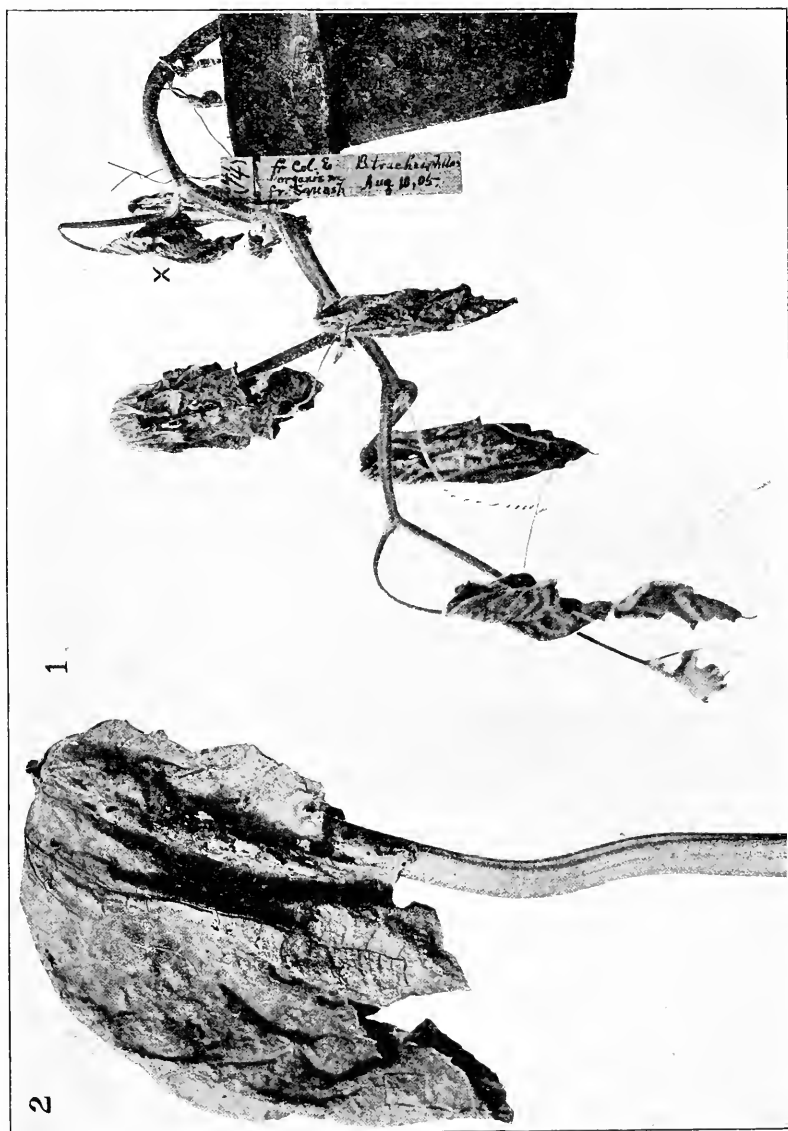


Fig. 54.\*

the natural manner of infection and has given very satisfactory results. These inoculations now number over 700. A great many check-plants were held for comparison and the number of accidental infections (when insect-carriers have been excluded) has been practically *nil*, whereas the number of successful inoculations in susceptible plants has frequently amounted to from 75 to 85 per cent of the total number punctured. In certain experiments (pages 246, 276) every inoculated plant has contracted this disease, which is one of the most infectious known to the writer. So many experiments have been made, under such a variety of conditions, and with such good success (except in case of the squashes already mentioned) that not the least doubt remains, either as to the bacterial nature of this disease or as to the particular organism which causes it.

\*FIG. 54.—Cross-section of small portion of a cucumber-stem attacked by *B. tracheiphilus* showing condition of one of the outer bundles. The pitted vessels lie in the more heavily shaded lignified part of bundle and only a very few of them are occupied by bacteria. All the spiral vessels are filled and the bacteria have formed conspicuous cavities in the primary vessel-parenchyma which is a living non-lignified tissue. Other tissues are uninjured. Drawn from a photomicrograph.



Wilt of Cucurbits.

Fig. 1. Cucumber-plant infected with a pure culture of *B. tracheiphilus* plated from the stem of a squash plant. Plant inoculated Aug. 10, 1905, by needle-pricks on blade of leaf marked X. Photograph made on Aug. 22. The vessels of the stem were plugged with a sticky white lacillus which was placed out. Surface of stem sound. About one-third natural size.

Fig. 2. Leaf inoculated with *B. tracheiphilus* by *Dialobes a. citrullus* night of Aug. 17, 1905. Blade shriveled in some places and wilting in others. A natural infection. Photographed Aug. '06, about natural size. For general infection of a plant inoculated in this way see vol. I, plate 23.





In the hothouse, the writer has succeeded in spreading this disease readily by means of leaf-eating beetles (*Diabrotica vittata*). Moreover, numerous field observations seem to indicate quite clearly that this is a common method of dissemination. Leaf-eating insects, and especially *Diabrotica vittata* (fig. 55), are, I believe, the chief agents in the spread of this disease. They feed readily, and sometimes the writer has thought preferably (fig. 7), on wilted leaves which are swarming with this organism. In this way their mouth-parts can not fail to become contaminated and to serve as carriers of the sticky infection. No other means of dissemination is known to the writer, and this is believed to be the common way in which the disease is distributed.\*

Seasonally the disease does not manifest itself until the leaf-eating beetles have put in their appearance, and this has led to the suspicion that the organism might pass the winter inside the bodies of these hibernating insects (*Diabrotica vittata*). As to this nothing definite is known. The greater part of the bacilli as they occur in bouillon are easily killed by freezing, but it is likely that some winter over in the vegetative form or in some more resistant form, in suitable places in the soil. The writer has attempted to plate the organism from the *Diabroticas* several times but always unsuccessfully, other organisms having speedily occupied the plates.

Possibly the squash-bug (*Coreus tristis*) is also responsible for the distribution of this disease, but the evidence on which the writer formerly made this statement does not seem to him as conclusive as it did, *i.e.*, the results obtained may be interpreted in another way, checks in sufficient number not having been made. The subject is open to further experiment, with the probabilities in favor of this bug being a carrier of the disease (see page 235).

The large lady beetle, *Epilachne borealis*, is a greedy feeder on squash foliage but I have not seen it feeding on the bacterially wilted foliage.

One experiment only was made with aphides, and this yielded negative results. Four cucumber-plants were sprayed thoroughly on both leaf-surfaces with one part of a potato-broth culture 2 days old diluted with three parts of water. On one of these plants 70 aphides (*Aphis gossypii* Glover) were colonized, and the other three were held as checks, all under bell-jars. The aphides (which were taken from watermelon-plants) crawled about on the wet surface and immediately began to puncture the plant in many places. In the end they injured it greatly, but no bacterial wilt appeared. This plant was under observation 32 days. Two of the check-plants remained free from the disease. The third was free from signs for the first 3 weeks, but lost all its leaves by wilt between the twenty-third and twenty-ninth day, and on examination of its stem at various levels the vessels were found

\*The above mentioned field observations were made by me several years ago. In the summer of 1905, accident enabled me to strengthen these conclusions. Several cucumber-leaves were inoculated late one afternoon in a hothouse containing about 20 well-grown plants. This house unknown to me contained a very few specimens of *Diabrotica vittata*, and next morning it was observed that the punctured parts of the leaves (those parts wetted by the bouillon culture) had been gnawed out by this beetle, while the remainder of these particular leaves remained intact. It was also noted that leaves on various other plants had been eaten somewhat during the night, and as a result their infection was anticipated. This inference proved to be correct. About a week or 10 days later numerous cases developed. In every instance the wilt began in the leaves bitten by the *Diabroticas* immediately after the date of placing the infectious material on the leaves which were punctured. The latter also contracted the disease. One of the plants infected by gnawings of the *Diabrotica* is shown in vol. I, plate 23. The strain used for these inoculations was that mentioned as non-infectious for the squash.

Two months later in a hothouse remote from the preceding (north part of the grounds U.S. Dept. Agric.) squashes were infected by needle-puncture with the strain already referred to as plated from a squash-stem and found infectious to cucumbers. Wilt of the leaves resulted. These wilted leaves were bitten by *Diabrotica vittata*, of which the house contained a very few only. On a side bench about 15 or 20 feet away stood 16 fine young cucumber plants which I had propagated for a second set of experiments with cucumbers. These were bitten at the same time as the wilting squash leaves, or soon after, and 15 of the 16 plants contracted the wilt within a few days, and in every case it began in the bitten leaves. The earliest (primary) stage of the disease in these insect-inoculated plants is shown in plate 15, fig. 2. In this case also there was no likely source of infection except the inoculated squash-leaves as there were no other cucurbits in the vicinity, and again the beetles were the carriers. Some muskmelons on the same bench were also bitten and inoculated by these beetles. Microscopic examinations were made, demonstrating the bacillus in the vessels, and typical poured-plate cultures were obtained.

In the 3rd set of squashes inoculated in 1905, after wilted areas had developed on the leaves, it was twice observed that these areas were the only parts bitten by the *Diabroticas* on those particular leaves. See also pp. 281, 282, and 284.

to be full of bacilli. That this diluted culture was virulent is also shown by the fact that out of 10 large cucumber-plants inoculated from it the same day by needle-puncture, 8 promptly contracted the disease. Up to this time therefore, the weight of the evidence favors the view that aphides do not play any part in the dissemination of this disease. Further experiments should be made. The fact that one cheek-plant contracted the disease in some unknown way shows that at least occasionally the disease may be induced by simple spraying in the absence of suctorial insects, and this is what invalidates the experiment with the squash-bugs. Some of the sprayed plants on which they were colonized contracted the disease, but the additional inference is of the *post hoc* sort.

The disease seems to be worse in moist, warm weather than in dry cool weather, at the same time excessively hot weather seems to be unfavorable to its spread. A soft watery condition of the tissues is believed to be favorable to the spread of this disease. In a number of instances it has been observed to do most injury in wet seasons, but it is not restricted to such seasons. Possibly, the greater injury during rainy periods is attributable chiefly to the greater number of infections, favored by cloud-screens and the moisture of the air. In a dry air many infected wounds probably dry out before the bacillus has secured a foothold, or are rendered sterile by sunshine. The bacillus is so well

distributed that if it were not for some such restraining circumstances it is doubtful if ordinary cucurbitaceous plants could be grown at all in the Northeastern United States.

Aside from suitable weather-conditions and the propagation of extra sensitive varieties, which should of course be avoided, the conditions most favorable to the spread of this disease, so far as yet known, are the multiplication of insect-depredators, particularly the leaf-eating beetles. Probably puncturing insects do less harm. Among growers of these plants there is, however, a widespread belief that *Coreus tristis*, the squash-bug, "poisons the plant," and this poisoning, as it is called, might well

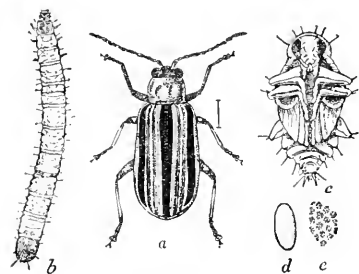


Fig. 55.\*

be the transmission of this bacillus. Further observations and experiments are necessary.

The extent of the vascular infection soon after the first secondary wilt supervenes was studied in plant No. 18 a diagrammatic sketch of which is shown in fig. 59. This plant was inoculated by needle-pricks on the blade of one leaf. The second day after secondary wilt appeared, the entire plant was fixed in alcohol. Subsequently, portions of this plant were infiltrated with paraffin, cut, stained and studied for the presence of the bacteria at the different levels indicated in the figure. These sections show that in the course of the 15 days which intervened between the needle-pricks on the leaf-blade at X and the fixing of the tissues in alcohol, the bacteria had penetrated into some portion of the vascular system of nearly every organ of the plant, the only exceptions being one lower leaf, certain tendrils, and a few centimeters of the undeveloped stem at the extreme top of the plant. This plant was inoculated October 1, 1894; wilt first appeared October 9 (in the pricked leaf); a trace of secondary wilt appeared October 14 and was well developed on October 15 in the 1st leaf above and the 1st below the inoculated leaf; on October 16 the plant was put into alcohol. When pricked the inoculated leaf was large and was near the apex of the vine. The infectious material came from vine No. 2.

The foregoing conclusions respecting the etiology of this disease are drawn largely from the following:

\*FIG. 55. —Stages in life history of *Diabrotica vittata*, the striped cucumber-beetle: a, mature insect; b, larva, c, pupa; d, egg; e, sculpture on egg. a, b, c, enlarged; d, more enlarged; e, highly magnified. After Chittenden. This beetle is the principal disseminator of *Bacillus tracheiphilus*.

## FIELD, HOTHOUSE, AND LABORATORY NOTES.

## EARLY STUDIES.

The work of the first few months was thrown away, principally because I did not know how to proceed, my technique being defective. Up to November 24, 1893, I had isolated five or six organisms as follows but had not obtained infections with any, and was very much at sea after a great deal of hard work:

- (1.) A slow growing white organism—on streaks and in poured plates. In the latter there were very numerous colonies but small even after months.
- (2.) A very rapidly growing white organism. It runs all over the plate in two or three days and is more or less dendritic. Spore-bearing—one oblong spore, central or at one end.
- (3.) A greenish organism which colors the agar. Much faster growing than 1 but slower than 2.
- (4.) An orange colored organism with crenate edges and in old specimens with radial fissures. It grows faster than 1.
- (5.) A faint pinkish growth.
- (6.) A rapidly growing, wrinkled organism, color dirty-Isabella.

The form 1 was undoubtedly the right organism, and probably the only one obtained from the interior of the plant. The others were undoubtedly intruders dragged in from the surface of the plant when I made my cross-sections, but I did not know it at the time. Subsequently I learned how to exclude outside organisms by the use of hot instruments. After that, labor was lightened, and inoculations with the right organism soon threw a flood of light over what had hitherto been an obscure subject.

The only genuine infections I had obtained up to this date were on several squash leaves by direct transfer as described below. A few earlier supposed infections on cucumber leaves obtained with the organism No. 6, did not progress beyond areas which had this organism plastered on them, and were undoubtedly not true infections but only suffocation spots due to the overwhelming mass of material used, *i.e.*, to defective methods of procedure with some soft-rot organism, or potato bacillus.

## SQUASH BLIGHT.

The following observations and experiments were made at Hubbardston, Mich., in 1893.

(1.) September 2: Several 9-foot vines became infected several weeks ago in the main axis, naturally, and have lost a dozen to 15 basal leaves by the blight. All the foliage is dwarfed and yellowish. The foliage shows wilt in the daytime with partial recovery at night, the squash plants being more resistant than cucumbers. One of the striking signs is the formation of a short branch in every leaf axis and the development of a flower cluster—often a dozen buds. One 9-foot vine bore 43 branches and several hundred flower buds. This strongly suggests what occurs in orange blight in Florida. The uninjured vines are very green and thrifty, 12 to 14 feet long. I know these vines have been infected several weeks, from the general appearance, which has changed only slowly in the last 8 days; from the old dried up appearance of the blighted leaves in the center of the hill; and from the case with which I get the milk-white bacterial ooze on the cut surface of basal branches near the main axis and also a foot or more away from it. This sticky ooze appears abundantly on the cut surface over the fibro-vascular bundles in as short a time as 2 to 4 hours when placed in moist air with the bottom of the inch-long segments in water. No such exudate appears on the cut stems of the healthy plants. September 11: The vines are still living and look as if they would live 2 weeks longer, but many leaves have died and all are yellow or dwarfed.

(2.) Four squash flowers were inoculated September 1, from the white ooze. The germs were thrust down upon the nectary and the mouth of the flower was tied up. August 30, two squash-flowers were so infected and 2 days later one was examined and the whole nectary disk found dead and one uniform colony of germs. September 7: Germus grew in the nectary; the stems were not infected, or at least no secondary signs appeared during my stay.

(3.) Bacteria from the cut ends of a squash-stem were pricked into two leaves (two vines), on August 30, using a sewing needle. Up to September 2, no blight. When pricked the blades of these leaves were 4 inches in diameter. Later: Typical blight appeared in both leaves in about eight days. (See under No. 10.)

(4.) August 30, a young squash-fruit was punctured in 40 places and many germs thrust in from several of the white colony-like bacterial beads on cut stems. The fruit oozed juice profusely. September 6: Squash still sound externally. On making sections through the flesh it looks water-soaked around the stabs for a breadth of nearly a millimeter. It is not rotten. Placed in moist air under a dish there is a moist sticky ooze from these water-soaked parts; not white; no ooze from other parts.

(5.) September 1: A leaf 7 inches across was wet on the upper surface over a square inch and a large white bacterial bead from the cut stem stirred up and pricked in with a needle. The leaf was then doubled together and inserted in a glass fruit jar (moist inside) and left 24 hours, the mouth being plugged with damp cotton wool (a defective method). September 2: Removed. No result where the germs were pricked in, but a sun-burn has appeared on the other half of the leaf. September 11, to a. m.: Two small wilted spots have appeared on the area which was pricked ten days ago; 6 p. m.: Spots have enlarged, each being about three-quarters of an inch long (each side of a vein) and not over one-fourth inch wide. The petiole of this leaf is 6 inches long and all leaves on the axis above and below are healthy.

(6.) September 1: A vigorous terminal shoot was enclosed in a large glass jar (wet inside and plugged with damp cotton wool), two leaves 3 or 4 inches wide, being first wet and several white masses of the bacterial ooze stirred into the water and then pricked in with a needle. September 2: Both infected leaves have blighted one-third to two-thirds and three others which touched them also show it. The blight includes the veins. These leaves are much younger and tenderer than in Experiment 5 (all probably due to sun-burn). September 11: Solely sun-burn.

(7.) Two ends of a vigorous squash vine were put into a wet glass can, two leaves on each being infected with bacteria brought from Washington. This growth was wrinkled, dirty Isabella color [the wrong organism]. The bacteria were teased up in water on the leaf and pricked in with a needle; two more were infected in the same way from another block of potato in the same Petri dish. Mouth of can was closed with cotton and wet rags. All 4 leaves blighted in 24 hours, but probably all was due to sun-burn. September 11: Solely sun-burn. No colonies appeared on cut ends of stem or petioles which were yellowing.

(8.) Some germs from the potato cultures [wrinkled dirty organism] were inserted into 2 green tomatoes and into the stem very thoroughly with needles. September 11: Tomatoes rotted slowly. Stem turned dark around puncture 1 mm. or more on outside and germs evidently infiltrated some distance into the tissue. My father afterwards picked and threw away the tomatoes for rotted, not knowing that I had inoculated them.

(9.) Germs from the very gelatinous Isabella-colored, wrinkled, colony [wrong organism] on potato were rubbed up in well-water and pricked into the parenchyma and veins of four large turgid squash leaves, both sides, pretty thoroughly, with a cambric needle on September 7, 11 a. m. Leaves several feet from ends, and marked with twine. No results.

(10.) September 7: The two turgid leaves (two different vines) which had bacteria pricked into them August 30, from the white ooze on cut squash-stems (see No. 3) are now badly wilted, while all the leaves to either side are turgid. These leaves are about 18 inches from the growing ends of the vines. I first detected the wilt yesterday morning (September 6), *i. e.*, about 8 days after the infection. They looked all right for 5 or 6 days and I had abandoned the experiments as hopeless and did not look at them for 2 days. The wilt to-day (September 7, 11 a. m.) is very decided and I can attribute it to nothing but the slow growth of the inserted germs. I now know all of the other supposed infections (*i. e.*, those obtained in moist air in 24 hours inside of glass jars) to be due in great part at least, and probably altogether, to *sun scald*. This was determined by getting the same results *without use of germs*. The cans rested on hot sand and the air became very hot inside and was saturated or nearly so with vapor of water.\* September 10: The two squash leaves wilted completely *but slowly*, and are now crisp dry. The petioles are still green and turgid, but one seems a trifle flaccid at the extreme tip although not yet shrunken or discolored. This one was cut away and divided into 0.75-inch segments, and put on end in moist air. Two hours later there were plain indications of bacterial ooze from the cut bundles on some of the segments, and at 6 p. m., *i. e.*, in 7 hours, all of the segments had each several beautifully distinct milk white bacterial beads resembling colonies. This was true even of the segments cut 3 or 4 inches below the blade of the leaf. This sets at rest all doubt regarding the possibility of inducing the disease by pricking in the bacteria taken fresh from the cut stems. September 11, 6 p. m.: Leaves to each side of these two are still perfectly healthy and no similar case of natural wilt at end of vines has appeared on any of the vines during the time I have been here. There is no shadow of doubt now as to what caused these leaves to blight. The striking thing is [was to me at that time] that the blight should have taken 8 days to develop. Only

\*Defective technique.

one additional vine has sickened naturally since my visit beginning August 22. This was all right until recently having only a few blighted leaves and withering petioles in the center of the hill, but now the whole of the big vine has wilted.

There was no decided change in No. 10 until September 14, p. m. (after a rain which occurred September 13). Then a whole leaf was found wilted suddenly. This was not the nearest leaf to the one which I had infected and cut away, but the next nearest, *i. e.*, the one on same side of the stem. This vine had been examined at 8.30 a. m. and found all right. At 6.30 p. m. this whole leaf had wilted. All the other leaves on the stem were upright. Turgid sections were cut from the petiole of this wilted leaf and put into a moist place over night and next morning they bore the milk-white drops on the cut ends. Other portions were put into alcohol. [These petioles were examined in February, 1909, in thin sections under the microscope and bacteria were found in the vessels].

September 15, 10.30 a. m.: Five additional leaves were found wilted on this stem near the original source of infection, two toward the center of the hill and three beyond the original source of infection 1.5 feet. Now the nearest leaf had wilted, *i. e.*, the one on opposite side of stem.

(11.) September 7: Many beads of the milk-white bacillus, which oozed from the cut end of squash-stems were stirred up in water and three large squash-leaves were pricked with a needle, not the one used for No. 9. These were tied with torn rags to identify them; No. 9, by white cotton twine. On September 14, this plant showed 3 wilted leaves.

#### POSSIBLE CARRIERS OF INFECTION OF *B. TRACHEIPHILUS*.

The following insects, identified for me by Mr. E. A. Schwartz, were found on diseased cucumber vines in 1893 and suspected by me at that time of being agents in the distribution of the wilt: *Diabrotica vittata* Fabr.; *Diabrotica 12-punctata* Oliv.; *Strigoderma pygmaeum* Fabr.; *Chauliognathus marginatus* Fabr.; *Epilachne borealis* Kirby (lady beetle); *Halticus uhleri* Giard = *H. minutus* Uhler (Hemipter); *Coptocycla guttulata* (not especially devoted to the cucumber).

#### INOCULATIONS OF SEPTEMBER 1, 1894.\*

One leaf on each of eight plants of *Cucumis sativus*, growing in the hothouse, was inoculated with bacteria taken directly from white beads oozing on the cut end of cucumber stems and squash-stems, the foliage of which was flabby or dying from the effects of the wilt-disease. A sterile steel needle was touched to the ooze from a cucumber-stem and twenty or thirty punctures were made in the center of the lamina of each of four healthy leaves. The needle was then flamed and an equal number of pricks was made on the blades of as many more leaves using bacterial slime from the ooze on a diseased squash-stem. Plants 1 to 4 were inoculated from the cucumber; plants 5 to 8 from the squash. The bacterial ooze from the cut cucumber-stems was so gummy and viscid that it could be drawn out on the end of the needle in a delicate thread over a foot long. The bacterial masses did not dissolve readily in water, not even after several hours, nor with vigorous crushing and teasing.

The temperature of the hot-house during the early part of the experiment was high, as the following records show: Sept. 5, maximum 100° F.; Sept. 7, at 1<sup>h</sup> 50<sup>m</sup> p. m., 99° F.; Sept. 8, at noon, 98°; Sept. 9, maximum 109°; Sept. 10, at 2<sup>h</sup> p. m. 104°; Sept. 11 at 9<sup>h</sup> a. m., 72°; noon 90°; Sept. 13, at 9<sup>h</sup> a. m., 72°, at noon 90°.

(1.) The first signs appeared the fourth day after inoculation and first in the pricked area. The sixth day the whole blade of this leaf was affected and drooping, and its apex beginning to dry out. All the other leaves remained healthy. The ninth day the blade of the leaf to each side of the pricked one was wilted. On September 11 the leaf-blade next above and the one below the pricked one were dry-shriveled, and the second leaf above showed change of color and wilt on one margin at the base of the blade. Twenty-seven hours later one-half of the blade drooped and had changed to that peculiar green characteristic of leaves wilted by the immediate presence of the bacteria. The other side of the leaf was expanded and turgid. The disease progressed more slowly after the plant was brought into the cooler laboratory (September 10). On September 13 the whole of the pricked blade was wilted. On September 15 all of the leaves were wilted, 5 above and 1 below the inoculated leaf. The sixteenth day after inoculation the whole stem was dry-shriveled except the hypocotyl which was still turgid.

\*Those who wish to have etiologic proof without following all of the inoculations are advised to read only those of July 16, 1896, beginning on page 276.

(2.) The first signs appeared the fourth day after inoculation, the pricked area being the first part to show the wilt. The fifth day the inoculated leaf was cut away at the extreme base of the petiole. The progress of the wilt had been that shown in the sketch (fig. 56). The wilt did not extend more than 0.25 inch below the pricks and the petiole was 2.5 inches long. It was hoped, therefore, that the bacteria were removed with the leaf and that the plant as a whole, would remain free from the disease. Such was not the case. On the twelfth day one or two leaves showed a slight tendency to wilt. The next day the wilt was more decided although the soil had been watered copiously. On the fourteenth day 6 leaf-blades were wilted and drooping, 1 below the removed leaf and 5 above. The sixteenth day the plant was dissected and its vessels found to be gorged with bacteria. All the leaves were shriveled, but the stem was still green and turgid. The bacterial ooze from the cut stem was viscid and strung out in delicate cobwebby threads when touched, the same as the slime from which the plant was inoculated. The organism was cultivated out of the interior of this plant and gave rise to a long series of cultures of *Bacillus tracheiphilus*. Stem, dried for herbarium. The cultures referred to were direct ones (Beef-broth tubes Nos. 1 and 2, September 17). These were feebly clouded on September 19, and looked alike. Each contained an actively motile bacillus. Tube 1 was used for inoculating plants 12 to 15; the organism was also cultivated out of it on slant agar, yielding typical colonies. Nine of these colonies when transferred to as many steamed potato cylinders

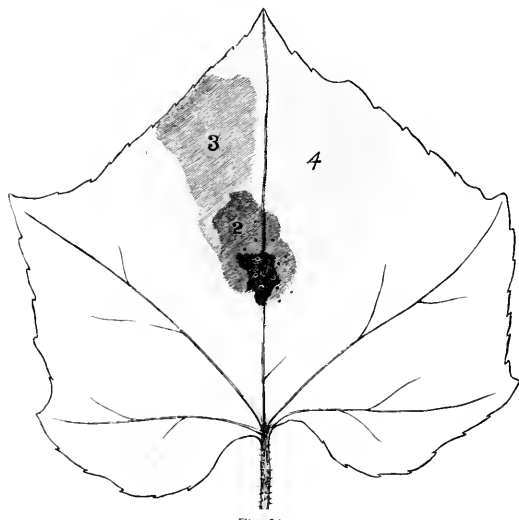


Fig. 56.

more than one-fourth inch below the pricks and the petiole was nearly twice as long as in No. 2. The disease was not cut out, however, by removal of the affected leaf. Like plant 2 the vine showed some signs of disease on the twelfth day and unmistakable ones the thirteenth day. On the fourteenth day 3 leaf-blades, 1 below and 2 above the removed leaf, were wilted and drooping. On the nineteenth day after inoculation the plant was about 80 cm. long. All of the leaves to the extreme tip had wilted. The stem was still normal in color and turgid except for a slight shrinking just under the insertion of the inoculated leaf. The vine was now cut and examined in many places. The bundles were gorged with bacteria, nearly every vessel being full of what seemed under the microscope to be one kind of organism. Part of these bacteria were motile. As in other vines of this series, the number of motile rods increased toward the tip of the plant, *i. e.*, at more and more

yielded in 6 days typical gray-white, wet-shining, thin, sticky growths, scarcely distinguishable in color from the potato itself; 6 other cultures were made on September 17 direct from the interior of this vine—3 slant agar and 3 potato. Two agars yielded nothing, the other, a single thin-edged, smooth, wet-shining, slow-growing, white colony (diameter 2 mm. after 15 days). The 3 potato tubes yielded typical growths of *B. tracheiphilus*, one of which was transferred to a tube of slant agar on September 23.

(3.) The pricked area was the first part to show signs of the disease, which it did on the fourth day after the inoculation (noon or earlier). On the fifth day at 3 p. m., the inoculated leaf was cut away at the extreme base of the petiole (the petiole was preserved in alcohol along with that of No. 2, and portions of the wilted parenchyma of each leaf). The inoculated leaf resembled that of plant 2 very closely, about one-third of the apical portion being wilted. The wilt did not extend

\*FIG. 56.—Leaf of *Cucumis sativus* (No. 2) inoculated with *B. tracheiphilus* by needle-pricks and shaded to show progress of wilt. First signs on fourth day (Sept. 5): 1, 6 a. m.; 2, noon; 3, 3 p. m.; 7, Sept. 6, 3 p. m. On this date the leaf was cut away at its base, but the bacteria had already passed down vessels of the leaf-stalk and had entered the stem as shown by subsequent events (petiole 2.5 inches long). Pure cultures yielding a long series of successful inoculations were afterward obtained from interior of the stem of this plant. Drawn by the writer.

remote distances from the point of inoculation. Examined in drops of sterile water many of those rods taken from near the tip were actively motile, while those taken lower down, *i. e.*, from vessels clogged solid with the bacteria, were not motile at all or only doubtfully so. The final condition of the bacteria in the vessels of the plant appeared to be a zoogloë stage. The organism was cultivated from the interior of this plant at various levels and found to be *Bacillus tracheiphilus*. In making these transfers the stems were shortened with a hot knife and the end dug into with a flamed needle. On September 20, under these conditions, the following transfers into tubes of sterile media were made from the interior of this plant:

(1) Four inches from the tip, Beef broth; (2) Do., Cucumber broth; (3) Six inches from the tip, Potato broth; (4) Do., Peptonized beef broth; (5) Eight inches from the tip, Beef broth; (6) Do., Potato broth; (7) Eleven inches from the tip, Beef broth; (8) Do., Cucumber broth; (9) Fifteen inches from the tip Potato broth; (10) Do., Cucumber broth.

One tube remained sterile (No. 2); 2 were contaminated (No. 8 with a pink organism and No. 10 with a white organism forming a pure white, wrinkled, fragile pellicle; 7 yielded moderately clouded cultures exactly alike and presumably all of them pure cultures of *Bacillus tracheiphilus*. The behavior of the organism in 5 of these cultures (Nos. 1, 3, 5, 7, and 9 was tested further by transfers to cylinders of sterile cooked potato. On this medium each one developed a thin, smooth, wet-shining gray-white, sticky slime, scarcely distinguishable in color from the surface of the potato itself, and perfectly characteristic as was afterwards learned, of the behavior of this organism on steamed potato.

(4.) The first sign appeared the fourth day after inoculation. The area in the vicinity of the needle-pricks was the first part to become flabby and discolored. Within a period of 4 hours, on the afternoon of September 5, the spot increased noticeably, being at least one-third larger at 4 o'clock than at noon. By the sixth day the wilt had extended on one side to the extreme base of the leaf-blade. The whole leaf drooped and two-thirds of it had changed color. The rest of the vine was normal except the tip of one leaf which was bruised in repotting. By the eighth day the whole leaf-blade had shriveled. On the eleventh day the blade of the leaf next above and of the leaf next below the inoculated one began to droop slightly and on the twelfth day they were entirely shriveled, while the next one above showed a tendency to droop. The thirteenth day the plant was photographed (see plate 16, fig. 1). There were then 3 shriveled leaf-blades and 3 freshly-wilted leaves further up the stem. At the top were three turgid leaves and at the bottom one. The fifteenth day the plant was wilted throughout except the stem and the base of some of the petioles which looked normal. There was no external indication of the cause of the disease. The stem was now cut open and examined under the microscope: 6 inches below the inoculated leaf every vessel of every bundle contained bacteria. Most of the vessels were gorged and large cavities had formed in the primary vessel-parenchyma of three bundles. The bacteria here were not clearly motile. Six inches farther down the vessels were still gorged but some of the bacteria were plainly motile. The tissue was less broken down. The bacteria also occurred an inch from the tip of the stem, but less abundantly. The vessels were full, however. The bacteria were also found in the petioles of the leaves where they were abundant. The disorganization of the bundles in this plant had proceeded further than in No. 8, examined the fourteenth day. Fourteen cultures were made from the interior of this plant as follows, the stem being cut with a hot knife, and its interior dug into deeply with a flamed, steel-needle and slime removed from the cavity:

(1) 3 gelatin rolls; (2) 5 gelatin stabs; (3) 2 beef-broth tubes (1 peptonized); (4) 3 tubes of agar (stab cultures). Two of the gelatin rolls remained sterile, one contained a mixed growth consisting of two small, white colonies, two yellow ones, and a mold spore. Two of the gelatin stabs appeared to be pure cultures, the others were contaminated. The two beef-broths clouded typically, but on microscopic examination they were found to contain round bodies as well as rods. The agar stabs all yielded typical, thin edged, gray-white, wet-shining surface growths. The round bodies in the bouillon cultures may not have been contaminations as they afterwards appeared in tube 1, September 17, made from vine 2. Also because a loop from one of these broths yielded a thin gray-white, wet-shining, typical culture when transferred to potato.\*

(5 to 7.) One leaf-blade on each plant was inoculated with bacteria from a diseased squash-stem. No result. The inoculated leaf did not wilt. Possibly the bacilli dried out in the pricks before they got a start.

(8.) One leaf-blade was inoculated with bacteria from a diseased squash-stem. The first signs appeared the fourth day after inoculation. The area in the vicinity of the needle-punctures was at

\*In July, 1909, from a primary natural infection on a cucumber (petiole), a non-infectious white coccus was plated out, *B. tracheiphilus* being dead. This coccus form is viscid and closely resembles *B. tracheiphilus* on potato, but its growth on agar, while smooth, is a denser chalkier white and it reddens litmus milk. It was stained by amy1 Gram. It did not grow in Ferni.

that time flabby and somewhat discolored. Some hours later there was a distinct increase in the size of the wilted spot. The sixth day the whole leaf-blade was affected. The rest of the plant was normal. The eighth day the leaf-blade was shriveled. The ninth day the blade of 1 leaf below and of 2 above the inoculated leaf showed decided wilt. The tenth day the blade of the first leaf below had changed to a lighter green in places (same color as the primary wilt). The second below was also drooping, two bright green leaves above had collapsed beyond recovery, and two more further up were beginning to droop. The eleventh day the plant was badly affected. Two leaves below the infected one and 3 leaves above it had wilted. By the twelfth day the plant had developed an advanced and very typical case of the wilt. Two leaves below the pricked one (there were no more leaves on this part of the stem) and 4 above it were shriveled beyond recovery and becoming dry. The one next above drooped to a slight extent and the next one very slightly. The thirteenth day the plant was photographed, all the leaves being wilted at that time (see plate 16, fig. 2). The fourteenth day the plant was cut to pieces and examined. Many vessels were clogged full of bacteria, a portion of which when examined in water were seen to be distinctly motile. Some had a darting movement half across the vessel. Most of the vessels in all the bundles were gorged with the bacteria. The organism was very sticky to the touch and would string out when touched with the platinum wire. Fig. 57 was drawn from a smeared cover-glass preparation of this sticky ooze stained with carbol fuchsin.

Of 6 gelatin tubes (roll-cultures) inoculated from this plant, 2 showed no growth, 2 were contaminated by a greenish liquefying organism, 1 by a white liquefying organism dubbed the "angleworm," and 1 by a cadmiun orange organism. Of 2 agar poured plates made from this stem, 1 contained nothing on September 18 and 1 about 50 colonies of a contamination—a gray-white, crenate-margined colony, wet-shining at the edge but the rest of the surface covered by a flour-like coating. This latter plate was made in an unusual way, *i. e.*, by crushing a segment of the clean stem (not externally sterilized) in a tube of sterile water and transferring a loop of this fluid to the agar. Three hypotheses occur as an explanation of these failures: (1) The parasite was dead in the particular part from which inoculations were made; (2) the organism was so viscid that it did not wash off the needle or dissolve readily in the melted agar; (3) the agar was too hot when inoculated, *i. e.*, exerted a killing influence [my technique was still imperfect].

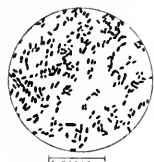


Fig. 57.\*

#### INOCULATIONS OF SEPTEMBER 13, 1894.

Plants 9 and 10 were inoculated September 13, 1894, from tube 1, September 8 (a potato culture from a cucumber fruit). The slime was flat, gray-white, wet-shining, in small patches on the center of the potato and growing slowly. It was very sticky and spun out in a fine thread when touched with a needle. No record of results was made or if made, it has been lost.

#### INOCULATIONS OF SEPTEMBER 19, 1894.

These inoculations were made on healthy but small hothouse vines of *Cucumis sativus* by means of needle-pricks. The infectious material was a beef-broth culture 2 days old (No. 1, September 17) derived from vine 2 and containing actively motile rod-shaped bacteria. The needle-pricks were confined to a small part of one leaf-blade of each vine.

(12.) The signs were so long delayed that no results were anticipated. Up to October 8 (p. m.) the plant was healthy in appearance, but by 9 a. m., October 9 (the twentieth day), the inoculated leaf-blade had changed color and begun to wilt. The blade and also the tip of the petiole were drooping at 2 p. m. The leaves at each side of the inoculated one were turgid. The twenty-first day the pricked leaf had begun to dry and hang down, its petiole being flabby. No other leaves showed any signs. The twenty-second day the next leaf below and the first one above the inoculated leaf were wilted and drooping—more at 1 p. m., than at 9 a. m. Three days later 7 additional leaves had wilted, all of them above the pricked one, making a total of 10 wilted leaves—*i. e.*, the one pricked, 1 below and 8 above. The twenty-seventh day the vine was brought into the laboratory. Segments, including a small fruit, were preserved in alcohol.

(13.) One leaf-blade was pricked. The plant subsequently dried up but not as a result of the inoculation.

(14.) No record.

\*FIG. 57.—Cover-glass preparation of *B. tracheiphilus* stained with carbol fuchsin. Smear made Sept. 15, 1894, with white ooze from cut stem of vine No. 8, which was inoculated Sept. 1 (plate 16, fig. 2). Organism motile in vessels.  $\times 1000$ .





Wilt of Cucurbits.

Hothouse-cucumbers inoculated with *Bacillus tracheiphilus* Sept. 1, 1894, by direct infection from cut surface of diseased stems brought in from a field, bacteria being introduced by needle-pricks on blade of one leaf.

- (1) Plant No. 4, inoculated with white sticky ooze from stem of cucumber. Photographed thirteenth day after inoculation—3 blades shriveled, 3 wilting, and 4 normal.
- (2) Plant No. 8, inoculated with white sticky ooze from a squash-stem. Photographed on thirteenth day when all the leaves had shriveled. Photographs about  $\frac{1}{2}$  natural size.



(15.) The pricked leaf was the first to show signs of the disease. They were noted the eleventh day after inoculation, but as the droop did not seem to proceed from any particular spot I was in doubt as to its cause. The thirteenth day the leaf above and the one below the inoculated leaf showed wilt. The fourteenth day they began to shrivel, the whole blade of the inoculated leaf was dry-shriveled, three additional leaves farther up the stem also showed a decided droop, and a fourth one, still higher up, a slight flabbiness. The rest of the leaves above and below were turgid and showed no sign of the wilt. The disease was moving up faster than down, as in some cases previously recorded. The following day (October 4) three additional leaves nearer the tip were wilted and one more toward the base making eleven in all, to wit: eight above the inoculated leaf and two immediately below it. The remaining basal leaf and the four leaves at the tip of the vine were still turgid. Two of the four noted as having wilted the previous day (the two nearest the point of infection) were then shriveling. The plant was now brought into the laboratory. The seventeenth day the wilt showed on the lowest leaf. All the leaves farther up as well as the stem in places had begun to shrivel. When segments of the stem were examined microscopically, the vessels were found to be full of the bacteria, which varied in size noticeably and looked much larger than usual (involution forms?). In the primary vessel parenchyma were many destructive lesions. The bacillus was also found in a small, green fruit, hanging midway on the stem and looking sound externally. Here it was confined to the bundles in the outer ring from which it slowly oozed on cross-section. The next day (October 7) the cut surface over the affected bundles was covered with a milky and very viscid bacterial slime which strung out on the tip of a needle a distance of 40 cm. (15.75 inches). The milky beads which oozed from the bundles of the cut fruit yielded a pure culture of *B. tracheiphilus*. On October 17 a potato cylinder inoculated October 7 was covered, except its edges, with a thin, gray-

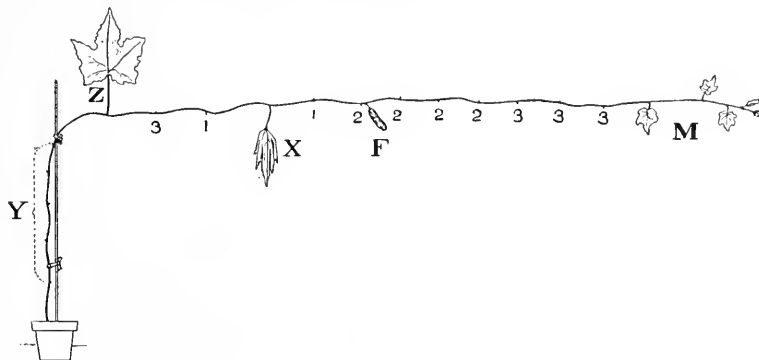


Fig. 58.\*

white, wet-shining layer which followed the irregularities of the surface of the steamed potato but was otherwise smooth, and was almost exactly the color of the potato but easily distinguished by its wet-shining surface. Thirteen days later there was no change in the appearance of this culture, except that it had spread over more of the potato. For the condition of this vine on October 4, see accompanying diagram (fig. 58).

#### INOCULATIONS OF OCTOBER 1, 1894.

Two sets of inoculations were made on *Cucumis sativus* in the hothouse. One plant (18) was infected with a gray-white, wet-shining organism from a potato culture of September 23 made from tube 1, September 17, which was inoculated from vine 2. The other two (16 and 17) were inoculated with a bacillus (examined in hanging drop), forming a cloudy growth in a fermentation tube of saccharose bouillon. This saccharose bouillon was inocu-

\*Fig. 58.—Diagram showing condition of cucumber-vine No. 15 on Oct. 4, 1894; x, leaf inoculated on blade Sept. 19 by needle-pricks. First signs of disease appeared on Sept. 30 in pricked leaf: (1) Position of leaves which showed secondary wilt on Oct. 2; (2) position of leaves which wilted on Oct. 3; (3) position of leaves which showed first signs of wilt on Oct. 4. Z and M, leaves which were turgid on Oct. 4. Z, which was the last leaf to succumb, drooped on Oct. 6. F, a small fruit from the interior of which *B. tracheiphilus* was obtained in pure culture. Y, lower nodes from which leaves disappeared naturally owing to small size of pot.

lated September 16 from a smooth, wet-shining, gray-white culture on potato (No. 1, September 8). This potato culture was derived from the interior of a cucumber fruit. On September 13 it was described as forming on the potato slow-growing, flat, gray-white wet-shining masses, which spun out in a fine thread when touched with a needle. The pricks were made with a steel needle on one leaf-blade of each plant, except No. 17, which was pricked on the center of the lamina of 2 leaves.

(16.) The first signs appeared the fifth day after inoculation, at noon, and first in the infected leaf. By 4 o'clock of the same day the disease had spread considerably and occupied about half of the leaf, forming a wedge-shaped area from the pricked portion outward. The rest of the foliage was normal. The eighth day the blade of the infected leaf was wholly dried up except a small area (1 X 1 cm.) at the tip of the petiole which was yet green but not turgid. The internode below was 12 cm. long. The next node above had lost its leaf long since (killed by a tobacco-water spray). The distance from the infected leaf to the second node above, which bore a good leaf, was 18 cm. Both of these nearest leaves as well as more remote ones were perfectly normal on October 9 (the eighth day) as was also the upright petiole of the pricked leaf. There was no further visible change until October 12. Then the first leaf below the pricked one drooped on one side. The first leaf above was still turgid. There was no change on October 13, 14, or 15. The last record of this plant was made on October 17. At that time the first leaf above the infected one was also drooping but all the others were normal.

(17.) The pricked leaves were well toward the extremity of the vine and were separated only by one internode. The leaves were normal at noon on the sixth day, but the morning of the seventh day both blades were drooping slightly and showed change of color. At 4 p. m. these signs were more decided and involved the whole leaf-blade. The following morning (October 9) the blades of the infected leaves were collapsed and had begun to shrivel. At 2 p. m. of the same day the nearest leaf to either side of the infected ones (leaves which were perfectly turgid in the morning) began to show unmistakable signs of the disease, *i. e.*, one blade was drooping decidedly at the tip and the other on one of the side lobes. The other leaves were perfectly healthy, and the petioles of the infected leaves were still turgid. The temperature on this day was 60° to 70° F. On October 10, at 9 a. m., the leaf next above the upper pricked one was drooping on both sides as well as at the tip and the next two above were flabby. The leaf next below the lower pricked one was drooping at the tip, and on either side. The second leaf below was still turgid, but by noon of the same day it was drooping. The tenth day all the leaves above were wilted and also the four next below the lowest pricked leaf, two additional ones having drooped that morning. The eleventh day the fifth leaf below the inoculated ones was drooping. The three below this were still turgid and were the only sound leaves remaining on the vine. The following day the fifth leaf down was wholly collapsed. The rest of the wilted leaves had shriveled but were not yet dry-brittle. The three basal leaves were still normal. The vine was now brought into the laboratory. On October 14 the stem had begun to shrivel in places and the uppermost of the three basal leaves had drooped, leaving only two sound leaves on the vine. Two days later all the leaves were wilted and the upper part of the stem was shriveled.

(18.) The blade of a large healthy leaf near the tip of the vine was pricked. On the afternoon of the seventh day the inoculated leaf was normal. The morning of the eighth day a part of the pricked leaf-blade had changed color and over half of it was wilted. By 2 p. m. of the same day it was wholly wilted with exception of a square centimeter where the blade joined the petiole. The petiole was turgid as was also the leaf to either side. The ninth day the pricked leaf was wholly soft-flabby, drooping and beginning to dry. All the other leaves were turgid and remained so for some days. The thirteenth day the leaf next above and the one next below the inoculated leaf were slightly flabby. Bacteria were now present in the vessels of the fruit. On October 15 the leaf to each side of the inoculated one showed decided wilt. Two-thirds of each blade hung flaccid. The rest of the vine was normal, including all of the petioles. The diseased leaves were now removed and put into alcohol. On the sixteenth day none of the leaves remaining on the vine showed any signs of wilt. Sections from all parts were removed and put into alcohol (fig. 59).

INOCULATIONS OF OCT. 25, 1894 (NOON).

Three young vines of *Cucumis sativus*, were sprayed thoroughly with a mixture which was three-fourths sterile water and one-fourth a potato-broth-culture of *Bacillus tracheiphilus* 2 days old (tube 8, October 23). These plants were placed under bell-jars in order that they might be kept free from aphides which I suspected might be the means of introducing the bacteria into the plant. Upon a fourth cucumber vine which was sprayed in like manner, numerous aphides (*Aphis gossypii*) were colonized, and the vine placed under a

bell-jar. The first three vines were used as checks on the behavior of the fourth. The air under the bell-jars was quite moist and at 4 p.m. water stood in tiny beads on the margin of the leaves. At the end of 24 hours the under surface of the sprayed leaves was still wet in places especially that of the leaf on which the aphides were colonized. Some of the latter had migrated to other leaves. All were sucking the plant juices and for fear of mechanical injury I brushed off and destroyed most of them. None were observed on the check vines. The bell-jars were removed and the plants exposed to the air for half an hour to dry off a little and then the jars were put back. This was done frequently during the experiment. The fourth day the vines were still healthy and the checks were free from aphides.

Tube 8, October 23, was inoculated from a very sticky potato culture (tube 8, October 17), which was inoculated from a single, small, white colony on a slant agar culture streaked September 27 from tube 1, September 17, which was inoculated from the interior of plant No. 2.

(20.) *Cucumber* (check). Plant about 5 inches high with two well-developed leaves and one more coming, also two green cotyledons. The bacterial fluid was sprayed on the under surface of the two largest leaves and the plant was then put back under the bell-jar. The eighth day after spraying, this vine was still healthy. It had grown an inch or two since October 25. It was still free from ants and aphides. Two days later it was healthy and growing rapidly. The twenty-third day the bell-jar was removed and not replaced as the plant was beginning to be spindling although no trace of the disease had appeared. The thirty-fourth day the vine was still free from the disease but had remained spindling since the removal of the bell-jar. By the fifty-first day the plant had lost all its leaves and the tip of the stem had wilted. It was not much over 1 foot high, having never recovered from the stunting due to keeping it under the bell-jar. Thin sections were cut and a microscopic examination made, but no bacteria were found in the vessels.

(21.) *Cucumber* (check). This plant was the same size as No. 20. It had two green cotyledons, bore one well-developed leaf, which was sprayed on its under surface, one twisted deformed leaf, and two undeveloped leaves. After spraying it was placed at once under the bell-jar. The eighth day this vine resembled the preceding in all particulars. It remained healthy and grew rapidly for a time but on the final removal of the bell-jar (the twenty-third day) it was beginning to be spindling although free from the disease. The thirty-fourth day it was still free from wilt but had remained spindling. The forty-sixth day it was brought into the laboratory and examined for the presence of the bacillus in its tissues. It had never recovered from the stunting due to keeping it under the bell-jar. Since the removal of the latter it had also suffered to some extent from mildew, from aphides, and on two or three occasions from insufficient moisture. It was not over 12 inches high. For the 3 weeks preceding it had been losing its foliage

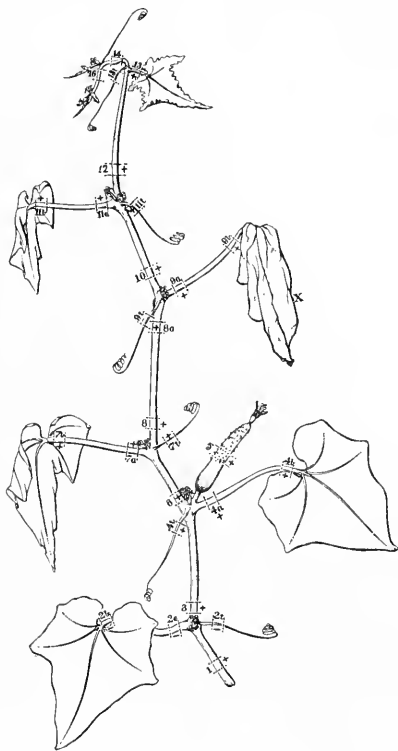


Fig. 59.\*

\*Fig. 59.—Cucumber No. 18, inoculated at x by needle-pricks with a pure culture of *Bacillus tracheiphilus* on Oct. 1, 1894. Numbered parts were removed and fixed in alcohol Oct. 16. They were subsequently embedded in paraffin, sectioned and stained for presence of bacteria, which were found in vessels at all points marked +, and not at those marked —. They occurred in greater or less numbers according to distance from inoculated leaf, or from main axis. Exclusive of wilt there were no surface indications of disease. About one-fourth natural size.

gradually and on this date (December 10) its last tiny leaf was found shriveled and also the upper 2 or 3 inches of the stem. Thin sections from the hypocotyl, and first, third, fourth, and fifth internodes, the last one of which was flabby, were examined under the microscope but there was not a trace of the bacillus.

(22.) *Cucumber* (check). Plant about 6 inches high, with two big leaves, two small immature leaves, and two green cotyledons. The three largest leaves were sprayed on the under surface and the bell-jar at once placed over the plant. The eighth day the vine was healthy and free from ants and aphides. It had grown about 3 inches since inoculation. Two days later it was nearly as tall again as when put under the bell-jar. The temperature in the hothouse that day (November 14th) was 83° F. The twenty-third day the bell-jar was removed. The vine was somewhat spindling but free from the disease. It was very warm in the hothouse when the bell-jar was removed, and part of the leaves wilted, the rest soon following in spite of careful watering. As soon as the wilting became noticeable the vine was repotted (November 18th) in a 4-inch pot and this probably hastened its collapse. On the twenty-ninth day the leaves were all shriveled and the stem was beginning to shrivel also. The latter was now cut at various heights, where it was still partly turgid, and examined microscopically. The vessels were found to be full of bacilli which varied greatly in size.

(23.) *Cucumber* (colony of aphides). Plant about 6 inches high, bearing two good leaves (blades about 2x2 inches), and two undeveloped leaves. The under surface of one of the larger leaves on which 20 aphides had been colonized October 24 was sprayed with the bacterial fluid until it was wet with mist and tiny drops. Fifty aphides, from a neighboring watermelon plant, were then placed on the wet surface, along with those already there, and the bell-jar replaced. Four hours later many tiny drops remained on the sprayed surface, one-fifth to one-sixth of its surface was still covered by these drops. Two of the aphides had moved to the upper (dry) surface, and 5 had crawled to the under surface of another leaf. The eighth day this vine was less healthy than the others. It had made very little growth owing to the aphides which were clustered on the terminal portion, causing it to be twisted and stunted. After removing the jar, to clean off the aphides and give fresh air, the plant had a drooping aspect. The sprayed leaf especially and one other, lacked turgidity and on close inspection I found that large areas of the leaves were pale green. Two days later, however, there was no sign of the flabbiness. The top was badly bent, twisted and stunted by the punctures of the aphides and the plant had elongated not more than an inch. The leaves, however, were turgid. The lack of turgor the eighth day was due probably to leaving the plant uncovered too long, the atmosphere under the bell-jar being nearly or quite saturated, while that of the hothouse was comparatively dry. The twenty-third day the bell-jar was removed permanently. The plant was spindling. Six days later (November 23), the vine was in a very bad condition although it was impossible to tell by inspection whether or not this was due to the multiplication of the bacteria in its vascular system. Three days later all the leaves on the growing tip had dried up and nothing was living and turgid except the pale green stem. The final slow death of the leaves was probably attributable to the direct effect of the aphides as the plant was badly dwarfed by their presence and had made but little growth. No signs of the bacterial wilt appeared, and on examination of thin sections I could find no bacilli in the vessels. They were entirely free from obstructions.

*Remarks.*—The manner of performing this experiment seemed to give every opportunity for infection through the ordinary stomata, since the under surface of the sprayed leaves remained wet over night. The results, however, do not bear out this hypothesis. This experiment also lends no support to the hypothesis that the bacterial wilt of Cucurbits may be spread by aphides. The entire lower surface of one leaf was sprayed thoroughly (wetted) with an infectious culture and the aphides which were colonized on it soon made numerous punctures but the plant did not become affected. Only one of the four plants contracted the disease and that was a check. The bacteria probably entered the latter through some fissure or other injury rather than through the stomata. Otherwise it is difficult to explain the immunity of the other three vines which were equally exposed to stomatal infection. It is, however, not known positively that infection never takes place by way of the stomata. This remains to be determined. Further experiments should be made also with aphides.

#### INOCULATIONS OF OCTOBER 25, 1894.

Ten old vines of *Cucumis sativus*, were inoculated in the hothouse from a potato-broth-culture (tube 8, October 23) of the gray-white, wet-shining, motile *Bacillus tracheiphilus*. The pricks were made with a sharp steel needle, in most instances on a single leaf-blade, but

once on a fruit. The record shows that in each case numerous delicate punctures were made, 40 in one case and probably from 40 to 50 in the others. The infectious material consisted of one part of the feebly clouded broth mixed with three parts of sterile water, *viz.*, a part of the same mixture that was sprayed on Nos. 20 to 23, just described.

The day temperature during the first 10 days of this experiment generally ranged from 65° to 85° F. but once it was as high as 90° (November 4, noon) and once as low as 50° (early morning of November 5).

(24.) The middle basal part of the blade of the fifth leaf from the tip was pricked. The vine showed no signs of the wilt until the morning of the eighth day. Then there was a wilted area about 1.5 cm. in diameter in the pricked portion. The rest of the leaf was turgid. By noon a V-shaped area extending from the pricks to the end of the leaf had wilted and by night the whole tip and one side had changed color and hung flaccid. The following day the entire blade of the pricked leaf had collapsed although its petiole was turgid. No other leaves showed signs at that time. The tenth day after inoculation the petiole of the pricked leaf was still turgid, as were also the leaves to either side of the pricked one, and a pistillate blossom in the axil of the inoculated leaf. The morning of the 12th day the extreme tip of the petiole of the pricked leaf was slightly flabby. By late afternoon of the same day the blade of the first leaf above the pricked one had wholly collapsed, except about 1.5 cm. around the apex of the petiole. There was no further change until the late afternoon of the following day. Then the blade of the first leaf below the pricked one was flabby and hanging down. The morning of the fourteenth day the blade of the pricked leaf was brown, the petiole was still green but flabby at the extreme tip. The small fruit in the axil was green and looked healthy. The second leaf down had lost its turgidity on one side. The second leaf above, which by reason of longer internodes was three inches farther from the pricked leaf than the second below, was still turgid. The vine bent in such a way that the second leaf down was 3 inches higher than the pricked leaf or any above it. The stem was green and turgid. At 3 p.m. of the fifteenth day the third leaf down was found to be flabby. No further records were made until 4 p.m. the nineteenth day after inoculation. It was then found that the disease had proceeded gradually farther and farther down the stem, wilting leaf after leaf until the seventh down had become flabby. In the morning of the same day this leaf was turgid. The morning of the twenty-third day the vine was brought into the laboratory and examined microscopically. The stem contained enormous numbers of bacteria, which did not seem to be confined to the bundles but to be out in the parenchyma to some extent as well. Part of the stem was shriveled but where the examination was made (4 to 6 inches below the pricked leaf) it was still green and turgid. All the foliage had been shriveled for some days. The upper part of the stem had also shriveled. The organism was cultivated out on November 17 into 4 tubes of potato-broth. On November 21, these four tubes were all alike, each being faintly clouded with rolling clouds on shaking. Potato-cultures were made from these tubes. On November 26, three of these tubes contained pure cultures of *B. tracheiphilus*; the fourth was contaminated by a yeast. The pure cultures on potato were thin, gray-white, wet-shining, and smooth, except that the layer was not thick enough to hide the coarsest undulations on the surface of the potato. The cultures were viscid to varying degrees (1 mm. to 6 cm.). The most viscid one (No. 2) contained the largest number of actively motile bacilli (one-half motile). The color of the slime was almost exactly that of the steamed potato itself.

(25.) The middle basal part of the blade of the fifth leaf from the tip was pricked many times. On the morning of the sixth day there were no signs. At 1 p.m. there was no change. At 2 p.m. there was a very faint change of color in the pricked area. This was scarcely noticeable. At 2:30 p.m. a piece of the leaf extending from the pricks to the apex had changed to a light green and wilted. At 5 p.m. a sketch was made showing the wilted part shaded. This was now a decidedly lighter green than the rest of the leaf (the change of color being much more noticeable than at 2 p.m.) and there was a slight flabbiness at the tip. The leaf, with the exception of that part shaded in the drawing, was turgid. The seventh day at noon the change in color (to light green) and the wilt were very typical, extending from the middle pricked portion of the leaf to its apex. The apex of the leaf was flabby and hung down. By 3 p.m. of the following day the whole blade of the pricked leaf had wilted. The following noon (the ninth day after inoculation) the nearest leaf each way from the pricked one had lost its turgor and was drooping a little. The petiole of the pricked leaf was turgid. At noon of the tenth day after inoculation the leaves to either side of the pricked one had wholly collapsed and the second leaf up was losing turgor. The second below was fully turgid. The petiole of the pricked leaf was still green but slightly flabby at the tip. The blade was becoming brownish. The morning of the eleventh day the second leaf above the pricked one was more flabby than on the preceding day and the third above had lost its turgor. The second below was flabby and the fourth below was

turgid. (The third down was wanting except the base of the petiole.) The next morning all the tiny leaves at the tip (beyond the second leaf up) had collapsed. By 5 p.m. of the same day the wilted leaves had begun to shrivel. The fourth down was still turgid. The fourteenth day the fourth leaf had lost most of its turgor. The petiole of the pricked leaf was still green but it was flabby nearly to the base. The blades of the first and second leaves below had shriveled, also those of all the leaves above the pricked one. The upper part of the vine was now removed for examination and cultures. The vessels were found to be full of the bacillus which strung out in fine gummy threads from the cut surface of the stem when a needle-tip was touched to it and withdrawn. The bacteria were exceedingly abundant and the inner tissues were considerably broken down. The organism was cultivated from this portion of the vine at different heights, inoculations being made from the stem into potato-broth from which a pure culture of *Bacillus tracheiphilus* was subsequently obtained on steamed potatoes.

The fifteenth day the fifth leaf down was flabby. The twenty-ninth day this plant was removed together with the other old cucumber vines, to make room for squashes. Dry material was saved from this vine for the herbarium. A futile search was made in it for spores of the bacillus.

(26.) The fifth leaf from the tip was pricked many times in one of the side lobes. At 10 a.m., October 31 there were no signs but by 1<sup>h</sup> 30<sup>m</sup> p.m. of the same day the pricked lobe had wilted. The first signs appeared, therefore, in this case at the end of the sixth day, the inoculations having been made in the afternoon. By noon of the seventh day the wilt and change of color had made marked progress in the pricked lobe and that portion of the latter in which the wilt first appeared had dried out. The temperature in the hothouse when this observation was made was 80° F. The following day (3 to 4 p.m.) the whole leaf-blade was flabby. The pricked portion was dry-wrinkled, the petiole

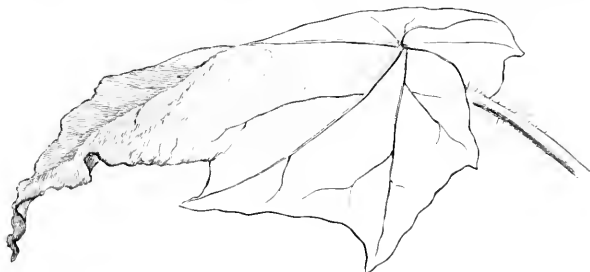


Fig. 60.\*

turgid. There was no further change until noon of the tenth day. Then the petiole of the pricked leaf was slightly flabby at the apex but still green. The blade was turning brownish on the pricked side. The nearest leaf to each side was turgid. At 4 p.m. the following day, the first leaf below had fully collapsed although turgid at 10 a.m. The second leaf above was still turgid. (The first leaf above was wanting, only the base of the petiole remaining.) The upper part of the vine hung down in such a way that the lower (collapsed) leaf was uppermost. The morning of the twelfth day the petiole of the first leaf below the pricked one was flabby. An observation made at 5 p.m. showed no further change. The morning of the fourteenth day after inoculation the blade of the pricked leaf was brown and dry-shriveled throughout. The petiole was still green and it was flabby only at the tip. The second, third, and fourth leaf down were flabby as was also the petiole of the second leaf. The blades of the fifth and sixth leaves below (all that remained) were flabby and shriveled including the petioles. This latter seemed anomalous. The petiole of the first leaf below was flabby and shriveled to its base although still green. It was in a much worse condition than the pricked leaf. Here we probably have to take into account the smallness of the pot and the age of the vine, the lower leaves being weaker than the others. The twenty-ninth day the plant was pulled up to make room for squash vines. A portion of it was saved dry for the herbarium. Unavailing search was made in it for spores of the bacillus.

(27.) The seventh leaf from the tip was pricked many times in the center of the lamina. There were no signs until the morning of October 31 (5 $\frac{3}{4}$  days). Then the pricked leaf was wilted in a V-shaped area opening outward from the pricks to the apex of the leaf. In the afternoon the condition was that shown in fig. 60. The apex was drooping. The rest of the leaf was normal. The following noon,

\*FIG. 60.—Bacterial wilt at 4 p. m., Oct. 31, on an inoculated leaf of cucumber plant No. 27. For condition 20 hours later, see fig. 61. Leaf pricked Oct. 25, 1904. First signs of wilt (darker shaded part) morning of Oct. 31. Drawn by Theodore Holm.



the whole leaf-blade was affected with the exception of about 1 cm. at the apex of the petiole (fig. 61). Over two-thirds of the blade was dry-wrinkling. The leaf succumbed very quickly. The eighth day after inoculation the pricked leaf-blade was almost wholly dry-wrinkled but the petiole was still turgid. The next noon the nearest leaf each way which showed only the faintest trace of want of turgor at 9 a. m. was flabby and drooping, especially the one below. The petiole of the pricked leaf was still turgid. The tenth day the petiole of the pricked leaf was still green but it was flabby half-way to the base. The shriveled blade was becoming brownish. The first leaf each way was more collapsed than on the preceding day, especially the lower one. The second leaves each way were still turgid. The eleventh day (10 a. m.) the second leaf below was fully flabby and drooping. The second above then showed only a very slight lack of turgor at the apex of the blade, but at 4 p. m. it was quite flabby and drooped. The twelfth day the petiole of the pricked leaf was green but flabby half-way to the base, the same as on the tenth day. The blades of the first, second and third leaf up were wholly collapsed and also the petioles of the first and second. The same was true of the blades of the first and second leaves down and the upper third of the petiole in the first leaf down. The third leaf below was still turgid. At 5 p. m. the blades of the first and second leaves below and the first, second, and third above were shriveling. The fourteenth day the blade of the pricked leaf was brown-shriveled. The petiole was still green but now flabby nearly to the base and shriveled half-way down. The third leaf down had begun to lose turgor. The petiole of the first and second leaf up were more flabby than that of the pricked leaf. The twenty-ninth day this vine together with



Fig. 61.\*

the other old cucumber-plants, was pulled out to make room for squashes. Dry material was saved for the herbarium. Search was made in it for spores of the bacillus, but none were found. (28.) The fourth leaf from the tip was selected for inoculation; the pricks were made near the apex of the leaf and were numerous. The first signs appeared on the morning of October 31 ( $53\frac{3}{4}$  days) in the pricked area. The pricked portion (the leaf had been purposely inoculated near the apex) was wilted and had become a paler green. Upward the wilting had extended to the apex of the leaf, 1.5 cm. beyond the pricked area, while downward it had extended only 3 mm. beyond the pricked area. The rest of the leaf was sound and turgid. The middle portion of the pricked area (earliest wilt) was brown. At 5 p. m. the wilted area had widened 2 mm. or more on each side but had not extended any farther down. The following noon the tip of the leaf had dried out and was hanging down. Both of the apical side lobes were now drooping. The basal lobes and middle basal part were turgid. The eighth day the pricked leaf-blade was wholly wilted and two-thirds dry-shriveled. The petiole was turgid. Drawings were made of this leaf in the different stages of wilt (see fig. 63). The ninth day (noon) the petiole of the pricked leaf was flabby at the tip and half-way to the stem, *i. e.*, for a distance of 2 inches. No leaves above were drooping. The nearest below was beginning to be flabby. The following noon the petiole of the pricked leaf was still green but flabby nearly to the base. The shriveled blade was becoming brownish especially in the pricked area. No leaves above were wilted and the first leaf below had recovered its turgidity during the night. The eleventh day at 10 a. m. there was no change but at 4 p. m. the first leaf below was flabby. The morning of the twelfth day the petiole of the pricked leaf was flabby to the base and shriveled two-thirds down. No further changes were recorded until the morning of the fourteenth day after inoculation. Then the petiole of the pricked leaf was shriveled to the base and hanging down limp. It was green only in the basal portion. The second leaf up was gone (removed by some one) and the third was flabby. None of the leaves below the pricked one had sound blades but there was a branch 8 inches long some distance below which was green and sound. The petiole of the first leaf below was flabby and shriveled two-thirds of the way to the base. The twenty-ninth day the vine was uprooted and dry material saved from it for the herbarium. Search was also made in it for spores of the organism.

\*FIG. 61.—Same as fig. 60, but 20 hours later, the only turgid part of the blade being at the extreme base.

(29.) The sixth leaf from the tip was pricked many times. The pricks were made along the midrib and in the mid-basal part of the blade. The first signs were noted at 10 a. m., October 31. Two-thirds of the pricked leaf hung down flaccid (that part beyond the pricked area). The rest of the vine was normal. The following day the inoculated leaf-blade had entirely collapsed and was hanging down. It was dry-shriveling but still green. The petiole was turgid. The eighth day the pricked leaf-blade was wholly dry-shrivelled. At noon of the ninth day the petiole of the pricked leaf was flabby and shriveled nearly to the base but was still green. The first leaf

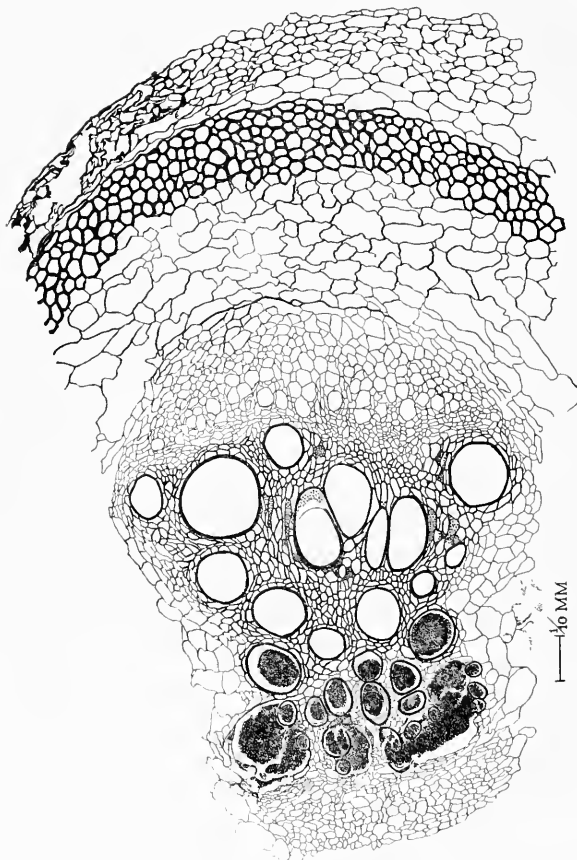


Fig. 62.\*

above had become flabby also. Twenty-four hours later the blade of the pricked leaf was wholly brown-shrivelled, and its petiole much as before, *i.e.*, shriveled nearly to the base. The second leaf above had become flabby and was drooping. The first leaf below, which was separated from the pricked one by a long internode, was still turgid. The eleventh day (a. m.) the second leaf above was wholly flabby and drooping. The tip of the third leaf above was also flabby and drooping. The

\*Fig. 62.—Segment of a cucumber-stem attacked by *Bacillus tracheiphilus*: Cross-section (inner phloem of an outer bundle to surface of stem) after fixing in absolute alcohol. All the spiral vessels are occupied, also two pitted vessels. Bacterial cavities in primary vessel parenchyma. Bacterial masses and softer tissues contracted by alcohol. Anacostia, D. C., July 21, 1903. Drawn from stained section with aid of Abbe camera. Slide 178-5.

first leaf below was now flabby on one side and the second below was drooping slightly and lacked its normal turgor on one side. In both cases the affected side of the leaf was anatomically nearest to that part of the stem which was in direct line with the insertion of the pricked leaf. By 4 p.m., the third, fourth and fifth leaves above were flabby. The first and second below were entirely limp and drooping. The third below was losing turgor at the apex. The morning of the twelfth day all the small leaves at the apex, above the fourth leaf up, were flabby but there were no further changes. At 5 p.m., the third leaf below was still turgid. The morning of the fourteenth day there was still a small portion of the base of the petiole of the pricked leaf which was turgid. The flabby portion just above this was yellowish. The blade of the third leaf below was now flabby. This was separated from the next leaf above by a long internode. Nothing now remained free from signs of the wilt except the stem. The upper portion of the vine was removed and taken into the laboratory for microscopic examination. The vessels were found to be full of the bacillus which strung out in fine gummy threads from the cut surface of the stems. A part of the vine was put into alcohol for paraffine

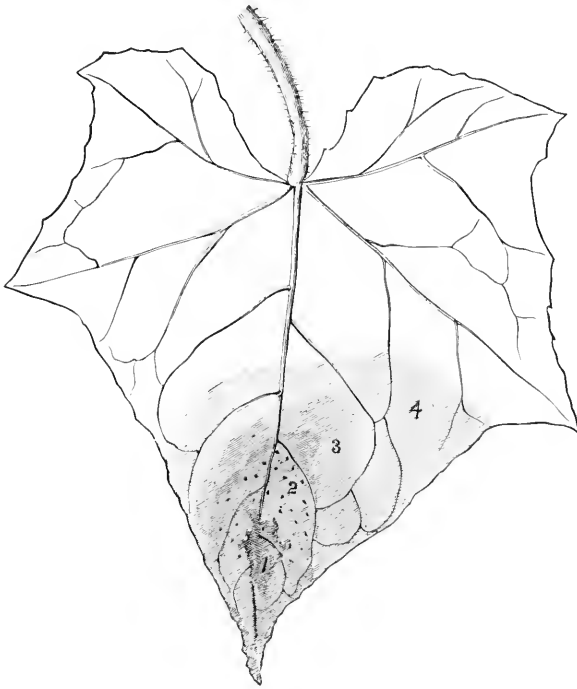


Fig. 63.\*

sections and the rest was saved dry to search for spores. The bacteria were exceedingly abundant in the stem and the inner tissues were considerably broken down. The organism was cultivated out and found to be *Bacillus tracheiphilus*. My method in this case was as follows: The stem was cut with a sizzling hot knife, a hole was then worked into the end 3 to 5 mm. deep, *i.e.*, below the burned surface, using a stiff, sterile steel needle. The stem was then squeezed a little, and fluid was transferred from the bottom of the moist cavity into sterile potato broth, using a freshly flamed small platinum oese. In this way four broth-cultures were made from the interior of this plant. Subse-

\*Fig. 63.—Inoculated leaf of cucumber plant No. 28, shaded to show gradual progress of wilt. Dots indicate needle-pricks. The first wilt appeared some time between fifth and sixth day after inoculation. At end of 5½ days (morning) 2 was wilted and 1 shriveling; on afternoon of same day 3 was wilted; the next morning 4 had wilted. By the eighth day the whole blade had wilted and two-thirds of it had shriveled. Plant inoculated Oct. 25, 1894. Drawn by Theodore Holm.

quently potato-culture No. 3, November 12, from one of these potato broths, yielded a thin, smooth, gray-white, growth, almost exactly the color of the surface of the steamed potato, but easily distinguished from it by its wet-shining appearance. This bacterial slime was viscid, stringing up 2 to 6 cm. when its surface was touched with a needle. Once I pulled the needle entirely out of the test tube before the gummy thread broke, and once from a cover-glass I stretched it up 20 cm. Examined in a hanging drop some of the rods were quiet, while others of the same form were actively motile. In other words, this potato-culture was an exact duplicate of No. 8, October 17, from which the broth was inoculated which served to infect this vine. The organism stained readily in carbol-fuchsin (1 to 3 min. exposure). The remaining part of the vine was left in the hothouse till the twenty-ninth day but no further developments were recorded. Dry material was saved for the herbarium. A futile search was made in it for spores of the bacillus.

(30.) The sixth leaf from the tip was pricked many times in one of the side lobes. There was no result. The pricked leaf remained thrifty although, by actual count, it had received forty pricks in one lobe. The plant was under observation until November 8 (14 days) and probably until November 23.

(31.) The twelfth leaf from the tip was pricked many times in the basal portion of the blade to one side of the center. By 10 a.m. October 31 the whole leaf had collapsed, changed color and was flaccid. It had already begun to dry out on the pricked side showing that the infection had come from that portion of the leaf. These were the first signs noted. The rest of the foliage was sound. The following day at noon the drooping leaf-blade was dry-shriveled except one basal lobe which was still flabby. The petiole was turgid. The next afternoon the blade of the pricked leaf was wholly dry-shriveled and the upper part of the petiole was flabby. The leaves to either side were turgid. The tenth day (noon) the petiole of the pricked leaf was still green, but flabby nearly to the base. It was not yet shriveling. The shriveled blade was becoming brownish. The rest of the foliage was still normal. There was no visible change until the twelfth morning. Then the petiole of the pricked leaf was flabby throughout and shriveled nearly to the base, but green. The leaves to each side, which were separated from the pricked one by long internodes, were still turgid. At 5 p.m. the first leaf below was flabby and drooping on one side (basal and middle lobes). The morning of the fourteenth day the blade of the pricked leaf was greenish brown (like No. 28). The petiole, shriveled nearly to the base, was still green but of a dull shade at the tip. The entire blade of the first leaf down had collapsed and had begun to shrivel at the edges. All the leaves above (7 in number) had collapsed and hung on limp petioles. They were still green. The twenty-ninth day the plant was pulled up and a part of it saved dry for the herbarium.

(32.) The eleventh leaf from the tip, *i.e.*, one well down on the old stem was pricked many times in the center of the blade. In a few days the leaves began to turn brown at the edges and by the sixth day the pricked leaf had browned and was almost entirely dried out but not from this disease. The plant had exhausted the soil in the 4-inch pot and done its life-work. Like many others of this planting it had probably ripened a small fruit. This vine was left to grow as long as the others but there was no result. The bacteria never reached the stem but were isolated from their necessary water-supply and destroyed in the browning leaf.

(33.) Many deep punctures were made in a small green fruit. The sixth day there were no signs of the wilt. The little cucumber was still green, for the most part, but there was a slight yellowing on one side, due probably to ripeness. It was still turgid and healthy. The foliage was normal. The tenth day after inoculation the little pricked cucumber looked as healthy as ever save for a water-soaked appearance around some of the pricks. There was no suspicion of rot and the water-soaked appearance might have been due to handling rather than to the bacteria as I saw similar appearances the preceding winter in Anacostia hot-houses on unpricked as well as pricked fruits. The blade of the small leaf from the axil of which the pricked fruit arose was normal the night before but was at this time flabby. Its petiole was turgid and also the leaves above and below. The next morning there was no change but at 4 p.m. the little leaf subtending the fruit had wholly collapsed and was shriveling. The first, second, third, and fourth leaves above the inoculated one were now flabby. These below were turgid. The vine had bent or trailed so that the former were below the fruit. The morning of the twelfth day there was no rot in the fruit but it was less turgid and yielded more under slight pressure than on the preceding day. The first and second leaves below (up in relation to the earth) were still turgid. The fourteenth day the tip of the vine was shriveling. A little white fungus which had undoubtedly gained entrance through one of the needle-pricks, now caused a small sunken place. The tuft of white hyphae was first noticeable the preceding day. The leaves below were not yet flabby except the one subtending the pricked fruit. The leaves above were shriveled. The upper part of the vine was now brought into the laboratory for microscopic examination. The vessels were found to be full of the bacillus which strung out in fine gummy threads from the cut surface of the stem and of the inoculated fruit. The bacillus was especially abundant in the latter. The inner

tissues of the stem were considerably broken down. The organism was cultivated out using the same method as in No. 29 and found to be *Bacillus tracheiphilus*. Samples were also saved in alcohol for paraffin sections. The following day the second leaf below was flabby. The twenty-ninth day this vine, together with the others, was dug up to make room for new plants. A portion of it was saved dry. Search was made in it for spores. Potato-culture No. 9, November 12 (from No. 9, November 8, which was inoculated direct from the interior of this vine) when examined November 28 was covered with a smooth, wet-shining thin gray-white slime, exactly typical of *B. tracheiphilus*. This was the most viscid culture seen. It strung up readily 30 to 40 cm., once 50 and once 53 cm. Examined in a hanging drop the rods were of variable length, but many short. All were about the same breadth, and none were motile. A cover-glass preparation was stained.

*Remarks.*—Signs appeared on four of the plants in  $5\frac{3}{4}$  days after inoculation. Two failed to take the disease, and signs appeared on the other four as follows: On two at the end of the sixth day, on one at the end of the eighth day or the beginning of the ninth and on one (that inoculated in the fruit) the tenth day. On October 31, the day on which six plants first showed the disease the temperature of the hothouse was  $72^{\circ}$  F. at 10 a. m. and  $80^{\circ}$  a few hours later.

The history of the culture used for making the foregoing inoculations is as follows:

- (1) Beef broth No. 1, Sept. 17, inoculated direct from vine No. 2, which was inoculated direct from a cucumber plant diseased naturally.
- (2) Streak from 1 on slant agar (Red x September 27).
- (3) Potato cylinder (No. 8, October 17) inoculated with one colony from margin of the preceding.
- (4) Potato broth (tube 8, October 23) made from 3.
- (5) Vine 25, etc., inoculated from tube 8, October 23.

#### INOCULATIONS OF NOVEMBER 2, 1894.

An attempt was made to discover whether squash-bugs and cucumber-beetles were the means of introducing this organism into the tissues of the plant. Cucumber plants (*Cucumis sativus*) were used for the experiment. Some of them were sprayed with a pure culture of *Bacillus tracheiphilus* and placed under bell-jars along with the insects. Other plants were not thus sprayed but were placed in insect cages into which the bugs or beetles were introduced after infecting them with the bacteria. Several attempts were made to infect some of the plants. The infectious material first used was obtained from a potato-broth-culture (tube 8, October 28) which was from slant potato-culture No. 8, October 17. The latter was the first sub-culture from a colony, the original source of which was the interior of vine No. 2. The potato-broth was faintly cloudy with rolling clouds when shaken and was full of an actively motile bacillus, as determined by examination of a hanging drop. The greater part of this tube was poured into about three times as much sterile water. For the cage experiments this diluted culture was sprayed on three pieces of cut squash-fruit each about  $2 \times 2$  inches so that the whole cut surface was wet. The sprayed squash was in three covered dishes. Into one dish I put the spotted beetle (*Diabrotica 12-punctata*). Into another I put the striped beetle (*Diabrotica vittata*) and into a third, a dozen squash-bugs (*Coreus tristis*). These insects were collected from a squash-field the preceding afternoon and were quite lively. After allowing them to feed upon and crawl over the infected squash-flesh for an hour, they were transferred to cucumber-vines inside of three insect-cages. This work was completed about 1 p. m. For the bell-jar experiments I used the same liquid, but the plants themselves were sprayed with it. These latter experiments (Nos. 35, 36, 39, 40) were begun about 3 p. m.

(34 a to c.) Five well-grown vines in three pots were placed in an insect-cage. The vines were 6 to 10 inches high and had 18 good leaves in addition to the cotyledons which had not yet withered. About 10 squash-bugs were taken from the sprayed squash-fruit already described and were put into the cage. Inside of the first half hour they began to stick their beaks into the vines and they were very particular to put them into the veins. The next morning they were not active. Only 3 were on the vines and these were not sucking. At 11 a. m. these bugs were taken out and put on pieces of squash freshly sprayed with the same fluid as on the preceding day. This fluid had remained in the atomizer and a microscopic examination showed that the bacilli were still motile. At noon the bugs were once

more introduced into the insect-cage. They were taken out at 3<sup>h</sup> 30<sup>m</sup> p.m. but not until I had seen several of them insert their beaks—always into the veins. The second day the vines were beautifully thrifty. Small diaphanous spots 2 mm. in diameter had appeared on several leaves. November 28, there was no trace of wilt. The twenty-seventh day one of the vines looked suspicious but no unmistakable signs of wilt appeared until the morning of the thirty-eighth day. Then one small leaf was wholly collapsed and part of its petiole hung flabby. The color and general appearance suggested the bacterial blight. Above and below this wilted leaf were turgid bright green healthy leaves. Three days later no additional leaves had wilted. If this plant was examined under the microscope records do not show.

(35 *a* and *b*.) A pot containing 2 vines about 8 inches high was placed under a big bell-jar. At 3 p.m. the leaves were atomized on both sides with the bacterial fluid and half a dozen squash bugs were turned loose under the bell-jar. The next morning the bugs were all on the pot, none on the vine. The second day the bugs were rather sluggish and not feeding much. They were removed the morning of the third day. The twenty-fifth day one leaf on 35*a* was freshly wilted and another small one was found shriveled down into the petiole and so must have become flabby some days before. Possibly this is the leaf referred to on November 9, as showing a tiny trace of wilt on one side of the lamina and of which there is no intermediate record. By the next morning the whole tip had wilted and hung flabby. Below was one good turgid leaf. The vine was small and had no other leaves. The following afternoon the last turgid leaf had wilted. The vine was now removed and brought in for microscopic examination. The vessels in the middle part of the stem were found to be full of the bacillus. In the hypocotyl, below the last wilted leaf, part of the vessels were still free from infection and in the others a portion of the bacteria were plainly motile. On the twenty-sixth day vine 35*b* was still normal. Nine days later (thirty-fifth day), a small leaf (about the third or fourth from the tip), which had been collapsing for some days, was wholly shriveled. The leaves above it were slightly flabby but as the plant had suffered considerably from mildew, aphides, and lack of water, I was uncertain how to interpret the phenomenon. I watered the pot well and awaited developments. The next morning 1 leaf below and 3 above had wilted, although the soil was moist enough. The plant was brought in and sections were made in various places. The vessels were found full of the bacillus and some of the rods were actively motile. In the vicinity of the first leaf to wilt the parenchyma of the stem also contained the bacillus, farther away the bacillus was sharply restricted to the vessels and in the hypocotyl even these tissues were free from it. Assuming the first signs to have appeared on December 3, the period of incubation was 31 days. The vine never recovered from the stunting due to keeping it under the bell-jar.

(36 *a* and *b*.) A pot containing two vines, 4 and 6 inches high, was placed under a bell-jar. It was sprayed like the preceding and half a dozen squash-bugs were turned loose upon it. The following morning the bugs were on the stem and ground and were not feeding. This was also true the second day. The bugs appeared to be sluggish. The twenty-sixth day the vines showed no signs of the disease. The thirty-fifth day, 36*a* which was very small and had been languishing for weeks, partly on account of the attacks of aphides, was quite dry with the exception of the base of the stem. The stem was now examined microscopically but no bacteria were found. On the thirty-fifth day 36*b* had a small leaf (about the third or fourth from the tip) which was wholly shriveled. It had been collapsing for some days. The leaves above it were slightly flabby, due perhaps to lack of water. Three days later vine 36*b* was badly shriveled with the exception of the hypocotyl and one or two of the first internodes. It was a small vine, never having recovered from its long sojourn under the bell-jar. It was brought in and examined microscopically. In the third internode above the hypocotyl the bacillus was abundant in a number of the bundles but motility was doubtful. In the first internode above the hypocotyl the vessels of one bundle contained plenty of the bacillus but they were not so closely packed as farther up and a part of the rods were plainly motile. No bacteria were observed in any part of the hypocotyl.

(37 *a*, *b*, and *c*.) Three pots containing 3 vines, 7 to 9 inches high, with 12 good leaves besides the cotyledons, were placed in an insect-cage into which 8 infected cucumber-beetles (*Diabrotica 12-punctata*) were introduced at 1 p.m. The latter were very active, flying away from the vines immediately to the top of the cage. The next morning the vines had sustained no injury. All the beetles had disappeared except three which lay dead on the ground and were being eaten by ants. Twenty striped cucumber-beetles (*Diabrotica vittata*) were now fed for half an hour on squash-fruit freshly sprayed with *Bacillus tracheiphilus* and put into this cage at 10 a.m. Half of them were removed at 3.30 p.m. and the rest were removed the next morning. Two of the 3 vines had been gnawed a little. All were beautifully thrifty. The twentieth day the inoculations were repeated using *Diabrotica vittata* which had been kept alive on squash-fruits for this purpose. These had been transferred to clean cut squash-fruits in clean covered dishes. (The old squash was moldy and rotten and mixed with dirt.) On them I sprayed at 11 a.m. the contents of a pure potato-broth-culture (tube 2,

November 20) diluted with four times its bulk of sterile water. The insects were allowed to feed until 1 p.m. Then the squash was removed and the beetles allowed to crawl over the bottom, top and sides of the moist infected glass dishes. At 4<sup>h</sup> 30<sup>m</sup> p.m. they were turned loose in the insect-cage. The next morning most of the beetles were at the top of the cage and had eaten but little. At 11<sup>h</sup> 30<sup>m</sup> I removed nearly all of the beetles and put them in dry, clean, glass dishes in order to starve them. They were left thus without food for 24 hours. I then sprayed the beetles and the top, bottom, and sides of the glass dish with the contents of a tube of potato-broth (culture of November 20) mixed with a tube of sterile potato-broth to which an equal amount of sterile water was then added. The potato-broth-culture was faintly clouded with rolling clouds when shaken. It was examined in a hanging drop and one in perhaps 20 to 50 of the rods on the edge of the drop were found to be motile. At 1<sup>h</sup> 30<sup>m</sup> p.m. the dish was uncovered in the insect-cage and the beetles turned loose again after wetting down the pots and sand on the bench, inside and around the cage. Four days later (the twenty-sixth day from the beginning of the experiment) there was no trace of the wilt. Some of the *Diabrotica vittata* were feeding very slowly but most of them not at all. On the forty-first day 4 out of 5 leaves on one of the vines were wilted, the lowest leaf and the upper leaves. These leaves had been all right in appearance the preceding day. *Diabrotica vittata* was still in the cage: 1 or 2 had begun to eat a little of late but most of them were hibernating.

On another plant in this cage the third and fourth leaves up yellowed and shriveled in December from serious gnawings but without suspicious signs. Sometime between January 1 and 4 the second leaf up wilted with signs regarded as suspicious. On the morning of January 4, the fifth leaf up showed a decided droop, although the earth was moist enough. Twenty-four hours later 4 small leaves above the fifth leaf were wilted. The stem was green and turgid but there were no healthy leaves on the plant. The stem was now cut open and examined in several places, but no bacilli were found, and the cause of the wilt of the leaves remained uncertain. Possibly bacteria might have been discovered in the leaves.

(38 a, b, c.) Three pots containing 3 vines, 6 to 8 inches high, with 12 good leaves besides the cotyledons, were placed in an insect-cage at 1 p.m. with 15 or 20 specimens of the infected cucumber-beetle (*Diabrotica vittata*). The beetles began to feed at once and all but one or two were taken out at 5 p.m. Holes had been gnawed in the leaves of each vine. The next morning the vines appeared normal, only a trifle gnawed. The second day the vines looked very thrifty. The twentieth day more specimens of *Diabrotica vittata* were sprayed with a pure potato-broth-culture (tube 2, November 20) and introduced into the cage. The next morning the beetles were at the top of the cage for the most part and had eaten but little. At 11<sup>h</sup> 30<sup>m</sup> a.m. I removed nearly all the beetles, starved them for 24 hours, sprayed them again (with potato-broth-culture of November 20—see 37a, b, c) and turned them loose in the cage as in the preceding experiment. The twenty-sixth day there was no trace of wilt. Most of the beetles were not feeding at all but some were eating slowly. The forty-first day there were still no signs.

(39 a and b.) A pot containing two vines, 16 and 17 inches high with 7 good leaves besides the cotyledons, were placed under a bell-jar after spraying both surfaces of each leaf. About 4 p.m. 6 or 8 cucumber beetles (*Diabrotica vittata*) were turned loose on them. The next morning all the beetles but two were removed. The leaves were considerably gnawed. One of the remaining beetles was removed the afternoon of the second day and the other the third morning. The twentieth day there were no traces of wilt. (No further record.)

(40.) One vine 4 inches high with 2 green cotyledons and 3 good leaves was placed under a bell-jar. About 20 specimens of *Diabrotica vittata* were introduced at 4 p.m. All went to the top of the bell-jar. The next morning all were removed. The leaves were riddled by bites. The seventh day the upper half of the upper leaf hung down flabby but without change of color. This leaf had been gnawed on both margins. Two days later (noon) 2 of the gnawed leaves showed very suspicious signs and the next morning the local wilt and change of color was unmistakable. Both leaves certainly had the bacterial disease. This vine had been sprayed with a pure culture of *Bacillus tracheiphilus*. The beetles were placed on it November 2, consequently the first unmistakable signs appeared in 9 days. On the tenth day at noon the leaves were more wilted. All the others were turgid although one was as badly bitten. At 4 p.m. a third leaf had changed color and wilted. This had been only slightly bitten. Evidently there were three distinct infections and perhaps more. On the eleventh day the three infected leaves hung down limp. On November 16 the vine had partly damped off at the surface of the earth. This was due to being watered too heavily the preceding day. (It was still under the bell-jar.) It had been going long enough to give striking results, however. The petioles were still turgid. On November 17, the vine was brought into the laboratory and examined microscopically. Bacteria were found in the vessels, and samples of the plant were saved in alcohol.

*Remarks.*—Up to December 10 only four good cases appeared and all were upon sprayed plants standing under the bell-jars. Three were on plants punctured by the squash-

bug and one was on a plant bitten by the striped beetle. The latter was the only case which came on with any promptness. In the others the signs appeared at such a late date that we must assume the introduction of an extremely small number of the bacteria, and possibly the same results would have occurred without the introduction of the squash-bugs (see notes on check plant No. 22). Records later than December 10 are wanting.

INOCULATIONS OF NOVEMBER 17, 1894.

One well-grown tomato-plant and 6 young squash-plants which were large for their age were pricked with a steel needle at 3 p. m., and inoculated with *Bacillus tracheiphilus* (cucumber-strain). Nothing could be more vigorous or apparently more disease-resisting than these young squash-vines. They were growing in 3-inch pots in the hothouse at the time of inoculation.

(41.) Tomato (*Lycopersicum esculentum*). A plant about 12 inches high, healthy and growing rapidly, was pricked on several leaflets of 3 leaves. Into one set of pricks I put bacteria taken from a potato-broth-culture of November 15. This was derived from slant potato-culture No. 1, November 12, which was made from slant potato-culture No. 8, October 17, already found by previous inoculations to be virulent (see Nos. 34, etc.). The second leaf received very sticky, actively motile bacteria direct from tube 1 November 12. The third leaf was inoculated with bacteria taken from a slant agar-culture (No. 12, October 28) which was from No. 1, October 23. This agar-culture was alive the preceding day as determined by its motility. The ninth day there was no wilt. The vine was growing rapidly and was exceedingly vigorous. It was now re-inoculated on 3 end leaflets of one of the leaves, from a colony (in the tube used for inoculations of November 26) in which more than half of the bacilli were actively motile, darting about in the water with great rapidity. The nineteenth day no signs had appeared. On this day (December 6) the vine was inoculated a third time, the part selected being a stout branch on the upper part of the stem, one which grew from the axil of the leaf pricked the ninth day. About 30 pricks were made, the needle being dipped into the culture each time. Sometimes the needle was thrust entirely through the stem. The culture used was a potato-broth (tube 2, December 3) in which the bacilli were motile. The twenty-third day this vine was making a magnificent growth. It had been inoculated three times but was pricked again on this date. The pricks, which were very numerous and which carried into the plant great numbers of sticky motile bacteria derived from potato-culture No. 5, December 6, were made on the main stem toward the top over a distance of 4 to 5 inches—both in the internode and the two nodes (which were less woody). A fifth inoculation was made January 3, using a great quantity of motile living bacteria taken probably from the slant agar culture of December 28. The needle was touched to the culture each time before using and 50 punctures were made.

On January 26, this tomato-plant into which I had pricked hundreds of thousands of living rods of *B. tracheiphilus* at various times during 2 months preceding, had made a strong growth and was entirely free from the wilt. It had outgrown the pot and the whole top was now cut away preparatory to repotting. The inoculation of January 3, like the others, caused no disease. The most that could be seen externally on January 26 was a slight shrinking and change of color to a duller green around the pricks, as if the bacteria had perhaps grown out into the tissue for a slight distance. The pricks themselves had enlarged with the increase in length and diameter of the stem and were bordered by a narrow ring of dry, dead tissue. Owing to growth of the plant the pricks were now about as long again as broad and, including the border of dead cells, most of them measured  $1 \times 0.5$  mm. The leaves near these pricks had remained healthy and the upper one had sent out a shoot from its axil since the inoculation. This shoot was 12 cm. long, and healthy. Beginning with the node out of which this shoot grew, and extending down 2 cm. there were 17 needle-punctures, all on the same side as the shoot and the nearest one within 4 mm. of its base. There was no evidence of any rot or softening around the pricked place externally or internally and a microscopic examination of unstained free-hand sections showed no bacilli in the tissues around the punctures. The branch into which the bacteria were pricked had shown no sign of disease and there was no change of color in the living tissue around the punctures, which were narrowly bordered by a band of white dead cells. This branch was preserved in alcohol. On March 12, there was still no trace of wilt.

(42.) Hubbard Squash (*Cucurbita* sp.). This vine, which showed the second true leaf, was infected near the tip and in the middle of one of the green cotyledons. Many pricks were made using a white, sticky, wet-shining bacillus from slant potato-culture No. 1, November 12 (cucumber-strain). The eighth day the cotyledon was yellowing in the pricked middle portion but without wilt. The next morning the yellowed area had increased in size. The tip seemed a little flabby but the wilt



was still doubtful. The eleventh day the pricked cotyledon was the only one out of more than a hundred which showed any yellowing. The following day the yellowing cotyledon exhibited a characteristic, wilted place in the yellow pricked area. This was about 1.5 cm. long and 8 mm. wide. The disease was progressing very slowly. The twenty-sixth day the pricked cotyledon had wholly shriveled. February 28 the vine was wholly dried out but no bacilli were found in the vessels or parenchyma of the stem.

(43.) Hubbard Squash. The first true leaf was pricked many times and inoculated with a white, sticky, wet-shining bacillus from potato-culture No. 1, November 12. The eighth day an irregular area including about 2 sq. cm. at the tip of the blade of the pricked leaf had wilted and changed to a light dull-green color. Certain pricks were inside of the wilted area. The following morning the wilted area had enlarged only a very little. The eleventh day the wilt was progressing very slowly. There was scarcely a larger area invaded than 3 days before. The twenty-sixth day the small wilted place first discovered had been dried up and brown for a long time. The disease had not spread. Six days later the leaf was brought into the laboratory and the portion which had wilted was put into alcohol. February 28 the vine had all dried out except the stem which was yellowish green and flabby. No bacilli were found either in the vessels or in the parenchyma of the stem.

(44.) Hubbard Squash. One of the green cotyledons was pricked and inoculated with bacteria from the potato-broth-culture of November 15. This was a pure culture and was actively motile on November 16 at noon. The eighth day no signs had appeared. The twenty-sixth day the pricked cotyledon was wholly shriveled. February 28 the vine was dried out completely. There were no bacilli in the vessels of the stem. The same day that this plant was inoculated, loops from the potato-broth of November 15 were transferred to other sterile potato-broths to see if the organism would grow. In 4 days there was typical clouding in these tubes.

(45.) Hubbard Squash. The first true leaf was pricked and infected from the potato-broth-culture of November 15. The eighth day an irregular patch including about 1 sq. cm. had wilted and changed to a lighter, dull-green color. This was on the margin of the leaf about 3 cm. to one side of the midrib. It included some of the pricks. The eleventh day the wilt was progressing very slowly. The invaded area was scarcely larger than 3 days before. The twenty-sixth day the pricked leaf was yellow and flabby on one side. Six days later the wilted portion was brown and dry. The disease appeared and dried out without spreading. The leaf was brought into the laboratory and the diseased portion put into alcohol. February 28 (one-hundred and third day) the vine had dried out with the exception of the stem which was still yellowish green and flabby. There were no bacilli in the vessels of the stem.

(46.) Hubbard Squash. One of the green cotyledons was pricked and inoculated from a thin, white, wet-shining growth on slant agar (tube 12, October 28 from 1, October 23). The eighth day there were no signs of the wilt. The twelfth day the pricked cotyledon was slightly yellow at the apex. The twenty-sixth day the pricked cotyledon was wholly shriveled. On January 26, (2 months after inoculation) the vine was stunted and had lost all of the lower leaves, probably because it was still in a 4-inch pot. The upper ones were green but small. The vine was brought into the laboratory and its stem was examined but no bacilli were found either in the vessels or parenchyma.

(47.) Hubbard Squash. Bacilli from a thin, white, wet-shining growth on slant agar (No. 12, October 28 from 1, October 23) were pricked into the first true leaf. The afternoon of the eighth day a small area of the pricked part of the blade looked wilted but it was somewhat doubtful. The next morning it was turgid. The vine was growing rapidly and was four times as large as when the bacteria were pricked into it. The eleventh day the leaf showed a very slight change of color in the middle of the pricked area, but I was doubtful whether this was the true wilt. In a day or two there was a tiny place radiating from the pricks ( $3 \times 8$  mm.) which was unquestionably wilted. The twenty-sixth day the disease had not spread. The thirty-second day the wilted spot was brown and dry. The disease had appeared and then dried up. It had not spread at all. The small spot was put into alcohol for future examination. On January 26 the vine was stunted and had lost all of the lower leaves. It was still in a 4-inch pot. The upper leaves were green but small. The stem was now examined but no bacilli were found either in the vessels or the parenchyma.

*Remarks*—The evidence derived from the inoculated tomato tends to show that the bacterial wilt of cucurbits is distinct from that of tomatoes, especially since 8 muskmelon plants and 1 cucumber plant inoculated on January 3 from the same culture as that used for the fourth inoculation of the tomato promptly contracted the disease.

The inoculated squash-leaves doubled in size between November 17 and November 22. On vines 43, 45, and 47 wilt spots appeared after a time in the inoculated part of the leaves,

but did not spread far and soon dried out. A wilt spot also appeared on at least one of the inoculated cotyledons (42). None of these infections led to any secondary signs. These results were all it will be recalled with the cucumber strain of the bacillus.

#### INOCULATIONS OF NOVEMBER 22, 1894.

Nine small vines of crookneck summer-squash (*Cucurbita sp.*), 8 to 12 inches in height, were inoculated by means of needle-pricks, using young pure-cultures of *Bacillus tracheiphilus* derived from three different plants (cucumber-strain). Three vines were pricked on one of the cotyledons, three on the blade of the first leaf, and three on the petiole of the latter. All of these were growing rapidly at the time of the inoculation. None of the leaves had reached their full size when pricked. Nos. 48, 49, and 50 were inoculated from tube 9, November 12; Nos. 51, 52, and 53 were inoculated from tube 3, November 12; Nos. 54, 55, and 56 were inoculated from tube 8, November 12. Tubes 9, 3, and 8 of November 12 were white, sticky, wet-shining potato-cultures looking exactly alike. These three cultures were derived from as many tubes of potato-broth which had been inoculated directly from the interior of diseased vines. No. 9 was obtained from vine No. 33, No. 3 from vine No. 29, and No. 8 from vine No. 25.

(48.) One of the cotyledons was pricked. The twenty-first day both cotyledons were shriveled but this came about naturally and was not the result of infection. Three months after inoculation the vine was wholly dried up but there were no bacteria in the vessels or parenchyma of the stem. Nos. 48, 49 and 50 were in the same pot.

(49.) This vine was inoculated on the blade of the first leaf. The twenty-first day the cotyledons had shriveled but there was no result from the inoculation. February 28, 1895, the vine was wholly dried up. Examination showed the vessels and parenchyma of the stem to be free from bacteria.

(50.) The petiole of the first leaf was inoculated. The twenty-first day the cotyledons had shriveled. There was no result from the infection. Three months after inoculation the vine was wholly dried up. It was examined under the microscope for bacteria but none were found, either in the vessels or parenchyma of the stem.

(51.) One of the cotyledons was pricked. The twenty-first day the cotyledon had shriveled naturally, and not as a result of infection. February 6, the vine was drying up. It was now examined microscopically but there was no trace of bacteria in the stem.

(52.) The blade of the first leaf was pricked. The cotyledons shriveled after a time and the vine dried up but this happened naturally and not as a result of inoculation. The stem was examined for bacteria on February 6, but none were found.

(53.) The petiole of the first leaf was inoculated. There was no result from the pricks. The cotyledons shriveled and the vine dried up after a time but the stem contained no trace of bacteria.

(54.) One of the cotyledons was pricked. There was no result from the inoculation. Both cotyledons shriveled and by February 1, the vine had lost all its leaves and begun to shrivel. On microscopic examination there was no trace of bacteria in the vessels.

(55.) This was inoculated in the blade of the first leaf. The cotyledons shriveled and the vine finally lost its leaves and began to shrivel but without any signs of the disease. There was no trace of the bacteria in any of the vessels of the stem where thin sections were cut and examined under the microscope.

(56.) The petiole of the first leaf was inoculated. The behavior of this vine was like the preceding. It finally lost its leaves and began to shrivel but no trace of the wilt appeared. No bacteria were found in the vessels.

*Remarks.*—This result was unexpected and discouraging. The squashes grew from seeds planted October 31. The cultures used were ten days old.

#### INOCULATIONS OF NOVEMBER 26, 1894.

Four squash vines and one potato vine were inoculated in the hothouse with a 6-day old culture of *Bacillus tracheiphilus*. A drop of sterile water was put on the surface of a squash leaf and a little mass of the white, sticky, wet-shining, actively motile (just examined) bacillus put into it, stirred around and pricked in with a steel needle. Each leaf received many punctures. The culture used (cucumber-strain) was slant potato No. 2,

November 19 (reinoculated November 21) from potato broth No. 2, November 17, which was inoculated from the interior of vine No. 24. The squashes were crowded, two together, in 4-inch pots.

(57.) Summer Crookneck Squash (*Cucurbita* sp.) This vine which was growing rapidly was pricked on the first leaf. The seventeenth day the cotyledons were shriveled but there was no result from the inoculation. The thirty-sixth day the vine had lost all its leaves and begun to shrivel. Sections were examined under the microscope but there was no trace of bacteria in the vessels of the stem.

(58.) Summer Crookneck Squash. The first leaf of a rapidly growing vine was pricked. There was no result from the inoculation. The cotyledons shriveled and after a time (36 days) the vine lost all its leaves and began to shrivel. No trace of bacteria was found in the vessels of the stem on microscopic examination.

(59.) Winter Squash var. Improved Hubbard (*Cucurbita* sp.). The first leaf was pricked. The vine was growing rapidly. There was no result from the inoculation. The seventeenth day the cotyledons had shriveled naturally. Two months after infection the vine was brought into the laboratory and the stem examined in several places. It was green and long and had lost most of its leaves except those toward the apex, where they were normal but small. There was no trace of bacteria. The plant was kept in a small pot.

(60.) Winter Squash var. Improved Hubbard. The first leaf was pricked. This vine behaved like No. 59. After a time it lost nearly all its leaves but no trace of the wilt appeared. The stem which was still green was examined in several places on January 28, but there was not a trace of bacteria.

(61.) Potato (*Solanum tuberosum*). This was a small shoot about 3 inches above the ground. Two small leaves were pricked. There was one other leaf lower down. Another shoot in the same pot was held as a check. The inoculation was without result. In three days the inoculated shoot trebled its size. The pricks were now open places and on both surfaces of the leaf there was a curious, pale greenish, ringed elevation surrounding each prick and consisting of an outgrowth of cells from the edge of the wound (fig. 64).

February 6, the vine was wholly shriveled. It was brought into the laboratory and examined for bacteria but none were found.



Fig. 64.\*

#### INOCULATIONS OF DECEMBER 6, 1894

Five potato, 6 tomato, 7 muskmelon, and 5 squash vines were inoculated in the hot-house with *Bacillus tracheiphilus* (cucumber-strain) from a motile potato-broth culture (tube 2, December 3). A small steel needle was used to make the punctures.

(62.) Potato (*Solanum tuberosum*). Two shoots of a plant 7 inches high were inoculated. On one the stem was pricked, on the other two leaves were pricked. The plant became diseased but not as a result of the inoculation. The foliage was stunted and finally became dry-shriveled. By the twenty-second day the vine was very sickly, both shoots equally so, and without apparent cause. The roots looked healthy and the disease did not seem to have spread from the pricks on stem or leaves. On microscopic examination no traces of bacteria were found in the tissues bordering the wounds. On this date the other inoculated potato shoots were twice as large and looked healthy.

(63.) Potato. Two of the three shoots of a vine 9 inches high were inoculated. One was pricked on the stem, the other on two leaves. For a time the vine grew rapidly but 40 days after inoculation the tops of the three shoots were dead and shriveling and none of the foliage was vigorous. This weakening had been gradual and had proceeded from the top of the shoots down and not from the pricked parts. The shoot pricked on the stem was now carefully examined microscopically at top and bottom but no fungi or bacteria were present. The pricked portion was sound. That the disease had not arisen from the inoculations was shown by several facts: (1) It did not begin in the pricked parts; (2) no bacteria were present in any part of the stem, at least not in that part which was badly diseased and wilting; (3) the shoot which was not pricked at all and the one which was pricked only on a leaf were as badly affected as the one pricked in the stem. Subsequently the plant was taken out of the pot, the dirt washed off and the root system examined. There was no sign of fungi or decay. The mother-tuber was not rotten or black and it had preserved its original form and appearance. Sections treated with chlorzinc iodide showed that most of the starch grains had

\*FIG. 64.—Diagrammatic section of a potato leaf, plant No. 61, showing new tissue formed about lips of needle-wound. Third day after inoculation with *Bacillus tracheiphilus*.

disappeared from the cells, not from all, however, as I should have supposed. The roots bore five small healthy tubers which seemed to be mature. The largest was 1 inch in diameter and the smallest 0.25 inch. In view of these facts the probabilities are that the plant had simply reached maturity, performed its function, and was dying naturally. Its early maturity was induced by the smallness of the pot. This explanation is the more probable from the fact that the roots had not made any attempt to occupy the new soil of the larger pot to which the vine was transplanted.

(64.) Potato. Two shoots, 6 and 12 inches high, were pricked, one on the stem, the other on two leaves. There was no result from the inoculation. The vine grew rapidly for a time, then the leaves began to yellow and shrivel from the top down, and by the forty-first day the upper part of the stem had shriveled also. Sections of the stem of the pricked shoot showed no bacteria. The earth was washed from the roots which were then examined. There were three small, mature tubers. The old one had not rotted.

(65.) Potato. Two shoots of this vine were pricked many times, one inoculation well-down on the stem, the other on 2 leaves. The shoots were 10 and 12 inches high. The plant grew rapidly for a time and then began to show signs of disease. The thirty-ninth day it had lost nearly all its leaves and the upper 6 inches of the stem had shriveled. Sections were made from the base of the stem and from the upper part close to the shriveled portion, which was far away from the point of inoculation, but no bacteria or fungi were found.

(66.) Potato. The plant bore four shoots, three 8 inches, one 10 inches high. Two shoots were inoculated. The tallest shoot was pricked on 2 leaves, and the other on the stem. After a comparatively short period of rapid growth, the leaves began to yellow and shrivel from the top down and finally the upper 8 inches of the stem shriveled (after 41 days). Sections of the pricked stem made below and at various places above and in the pricked portion were examined under the microscope critically. No fungi or bacteria were present or at least none were sufficiently abundant to be detected in unstained sections. The other stems in the same pot were equally affected. One of these was not inoculated at all. Clearly the change was not due to the insertion of *Bacillus tracheiphilus*. On washing away the earth from the roots the cause of the decline was evident. The plant had matured five small tubers and finished its life work. The old tuber was not rotten.

(67 a and b, 68, 69 a, b, 70.) Tomato (*Lycopersicon esculentum*). These 6 plants were 6 to 8 inches high. Each was given many pricks, some in one leaf, others in the stem. There was no result from the inoculations.

(71, 72.) Muskmelon var. Miller's Cream (*Cucumis melo*). These vines were 3 inches high. One leaf on each was pricked many times. No result.

(73, 74.) Muskmelon var. Extra Early Hackensack. Same size as preceding. One leaf of each vine was pricked many times. No result.

(75.) Muskmelon var. Extra Early Hackensack. One leaf of this vine was pricked. The eighth day there were slight indications of wilt at the tip of the pricked leaf. Twenty-four hours later half of the pricked leaf was flabby and hanging down. The twelfth day the first and second leaves above the pricked one had collapsed. The plant was not over 3 to 4 inches high. The following morning the vine was wilted. It was brought into the laboratory and examined under the microscope. Bacilli were present in the interior of the plant in large numbers. Material was preserved in alcohol.

(76.) Muskmelon var. Extra Early Hackensack. One leaf was pricked. No result.

(77.) Muskmelon var. Extra Early Hackensack. A small plant like 75. One of the leaves of this vine was pricked. The seventh day after inoculation an irregular wilt spot (about 0.5 sq.cm.) had appeared on the side of the leaf toward the apex and within the pricked area. Two hours later it had spread considerably and now involved about a dozen pricks. The ninth day the whole of the pricked leaf was flabby and hung down, also the next leaf above. (The vine was small and these two leaves were close together.) The twelfth day the pricked leaf, the first leaf above, and the tip of the vine were wholly shriveled. The following morning the whole vine was wilted. It was brought into the laboratory and examined microscopically. Large numbers of bacilli were found in the bundles, some of which were actively motile. Material was preserved in alcohol.

(78.) Winter Squash var. Hubbard (*Cucurbita* sp.). A leaf 3 inches broad was pricked many times at the apex. Nearly 2 months after inoculation (January 25) when the last leaf had shriveled the vine was brought into the laboratory and examined. The plant was stunted by being in the same pot with a larger vine. Aphides and mildew had begun to attack it. There was no trace of bacteria in the vessels or parenchyma of the stem.

(79.) Winter Squash var. Hubbard. The apex of a leaf 3 inches broad was pricked. This vine proved very resistant for a time but finally began to shrivel. On February 27 it was wholly dried out. It was not observed very carefully during the two or three weeks immediately preceding this date. This vine was now brought into the laboratory and examined microscopically. The vessels of one

bundle contained bacteria (small bacilli) in large numbers. Higher up one or two vessels contained bacilli of much larger size and in smaller numbers. Some of the vessels also contained a branching mycelium suggestive of *Fusarium*. The pot stood on a bench where watermelon wilt experiments were carried on previously.

(80.) Winter Squash var. Hubbard. The blade of a leaf 1.25 inches broad was pricked many times at the apex. Two months after inoculation the vine was brought into the laboratory. All but a few upper leaves were shriveled. The stem was still green and turgid. The latter was examined in three places for bacteria but none were found.

(81.) Winter Squash var. Hubbard. A leaf 4 inches broad was pricked many times at the apex. No result. Owing to crowding in a small pot only the upper leaves were alive on January 28, and these were dwarfed. The green stem was examined for presence of bacteria, but none were found.

(82.) Winter Squash var. Hubbard. A leaf 4 inches broad was pricked many times at the apex. February 28 the vine was wholly dried out. No bacteria were present in the vessels or parenchyma of the stem.

*Remarks.*—None of the potatoes or tomatoes took the disease and only two of the muskmelons. The squashes proved very resistant. They were in 4-inch pots and grew well for some time after inoculation. No. 79 was not affected at first but seemed to be affected after a long time. On December 20 the squashes were still in 4-inch pots and growing satisfactorily.

The early maturity of the potatoes undoubtedly resulted from keeping the plants too long in small pots. All the potatoes in the hothouse behaved in the manner described irrespective of whether they were inoculated or not. The tubers were planted in November and the shriveling began in December and on January 14 was apparent on all but two plants. Several examinations showed no bacteria or fungi in the tissues and the plants had not suffered from aphides or red spiders, nor had they been neglected or frosted. They were in 4-inch pots for about a month when they were transferred to 6-inch pots (about January 4) to see if this would help them to recover. It did not, however. When repotted most of the vines were over 2 feet high. These facts favor the supposition that the yellowing and shriveling was a natural one, occurring after the plants had performed their life-work, which was hastened by the small size of the pots.

From this experiment it was evident that the disease could be produced in the muskmelon with bacilli taken from the cucumber, but not with certainty in squashes.

The temperature in the hothouse the first two weeks after inoculation (December 6 to 20) varied from 60° to 90° F.

#### INOCULATIONS OF DECEMBER 10, 1894.

Fifteen plants including 3 hyacinths (*Hyacinthus orientalis*), 2 Hubbard squashes (*Cucurbita* sp.), 2 summer crookneck squashes (*Cucurbita* sp.), 1 potato, 2 cucumbers, 4 tomatoes, and 1 cantaloupe, were inoculated in the hothouse, with a white wet-shining, sticky, motile bacillus (cucumber-strain) growing on a potato-cylinder (pure culture No. 5, December 6, from potato-broth No. 2, December 3). The greatest pains was taken to do the work thoroughly. After each plant was thoroughly pricked I went back over the bench and pricked them again. Much material was used in pricking which was done in the afternoon. More than half the bacteria in this culture were motile. The temperature of the hothouse from December 6 to 20 varied from 60° to 90° F.

(83a, 84, 85.) Hyacinth. These plants had been potted 3 days at the time of inoculation and the green bud had pushed up 1 to 2 cm. In each 15 or 20 needle pricks were made into the bud, some of them deep. Up to the eighteenth day there was no sign of the blight.

(86.) Hubbard Squash. At the time of inoculation this vine was about 2 feet high and had 6 good leaves. It was very thrifty. About 40 pricks were made on the apex of the blade of the third leaf which was about 5 inches broad.

Up to February 27 (79 days) the vine showed no trace of the wilt. The stem was then examined in several places but the vessels and parenchyma were free from the bacteria.

(87.) Hubbard squash. This was a very thrifty vine, about 16 inches high, with 6 good leaves. About 50 pricks were made on the middle and apical part of the blade of the fourth leaf which was about 4 inches broad. The eighteenth day there was no sign of the blight.

On February 27 the vine was entirely dried up but an examination of the stem showed no bacilli in the vessels or the parenchyma. The Hubbard squashes were planted October 31 and were in small pots.

(88.) Summer crookneck squash. This vine was about 1 foot high and had 4 good leaves: 75 pricks were made in the blade of the second leaf which was about 2.75 inches broad.

The wilt did not appear. February 27 the vine was wholly dried up but no bacilli were found in the vessels or in the parenchyma.

(89.) Summer crookneck squash. This vine was about a foot high and had 4 good leaves: 60 pricks were made on the blade of the second leaf which was about 2 inches broad. February 6 the vine was nearly dried up. There was not a trace of bacteria in the stem. Three shoots in a 3-inch pot sufficiently explains the early death of the plant.

(90.) Potato (*Solanum tuberosum*). A shoot about 16 inches high was selected for inoculation. Many pricks were made in the middle and upper part of the stem over a distance of about 8 inches. Two leaflets were pricked many times also. No sign of disease appeared on shoot or leaves.

On February 6 the vine was brought into the laboratory and examined. It was still green and there was no trace of bacteria in the stem.

(91.) Cucumber (*Cucumis sativus*). This vine, which was about 16 inches high, was rather badly mildewed at the time of inoculation and some aphides were present. The first and second leaves had shriveled from the mildew. Six good leaves remained. The blade of the fourth leaf which was about 2.5 inches broad was pricked many times. At 11 a. m. the eighth day after the inoculation there were no signs of the disease, but at 9 a. m. the following day the wilt was of such an extent and character that it must have appeared soon after the previous day's observation. The affected leaf was cut off close to the stem to see if in this way the plant could be prevented from taking the disease. The length of the petiole was 4 cm. The blade was 6 cm. long by 7 cm. broad. The wilt was in an irregular wedge-shaped piece (the pricked area) broadening toward the apex (fig. 65). It extended in the vicinity of the midrib, to within 1.5 cm. of the tip of the petiole. Fourteen days after inoculation the second leaf above the pricked one was wilted and the following day the first leaf above and the first below the pricked one were wilted and shriveling. The first constitutional signs were 5 days

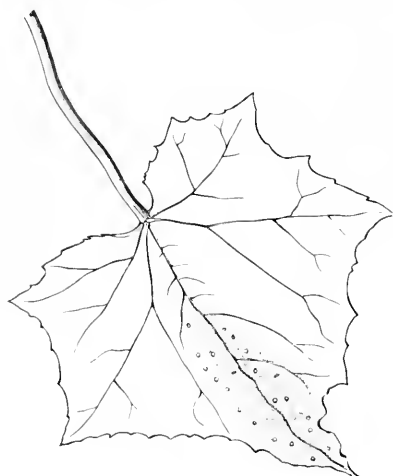


Fig. 65.\*

after the removal of the pricked leaf. On the sixteenth day the last leaf collapsed. The day following the stem was still green and turgid. It was 43 cm. high and had 10 internodes. The vine grew from a seed planted September 21. It had been kept in a small pot (4 inch) along with several other vines and was also dwarfed from the presence of mildew (*Erysiphe cichoracearum*) and aphides. There were blossoms from each node. The diameter of the first internode (hypocotyl) was 3.2 mm. That of the sixth (just below where the inoculated leaf was cut away) was 2 mm. All the leaves were now dry-shriveled. Portions of the stem were saved dry in an envelope for future examination and portions were put into five small vials of alcohol as follows: (1) Base of pricked leaf and portion of internode above and of one below: The vessels of each bundle were gorged with the bacillus and the tissues were somewhat broken down. There were scarcely any motile bacilli. Only after a long search could I find any whatever and then only a very few. (2) Middle part of the fourth internode: The vessels were gorged with bacilli, although the tissue was apparently sound; there were not many motile rods, although a larger number than in the section in vial 1. (3) Third internode (not examined). (4) Second internode: The vessels of one bundle were gorged but those of the other bundles were nearly free, some entirely so (?); a good many rods were darting and tumbling about, more than in the fourth internode. (5) Middle of first internode (hypocotyl): None of the vessels were clogged; some appeared to be free, others to have a few scattering motile rods.

\*FIG. 65.—Leaf of cucumber plant No. 91 (see text). Drawn by Theodore Holm.

(92, 93, 94, 95.) Tomato. Vines 8 to 10 inches high and pricked many times, 2 in stem and 2 in the blade of one leaf.

No results.

(96.) Cucumber (*Cucumis sativus*). This was a vine about 10 inches high with three good leaves. The uppermost one was given many pricks. By the seventh day (9 a.m.) this vine had contracted the wilt very decidedly on the terminal (pricked) portion of the leaf (apical two-thirds of the blade). The preceding day there were no signs of the disease. The wilted portion of the leaf hung down flabby while the rest of the plant was turgid and healthy except for a little superficial mildew. It had taken six days for the disease to develop. It was plain from the different aspect of various parts of the wilted portion that the wilt began in a V-shaped apical portion within the pricked area. By 2 p.m. the leaf blade was wholly flabby. The following morning the petiole was still rigid and normal. At 9 a.m. of the eleventh day the whole of the small top above the pricked leaf was wilted. This top was normal in appearance at 5 p.m. of the preceding day. The leaves below were still turgid as was also the case at 1<sup>h</sup>30<sup>m</sup> p. m. of the same day. The twelfth day (9 a.m.) the first leaf below the pricked one was wilted. It was normal at 5 p.m. the preceding day. The following noon the first leaf below had begun to dry out and the second leaf down was flabby.

The fourteenth day the vine was brought into the laboratory and thin sections from the lower part of the first internode below the pricked leaf were examined. The lower portion of the internode was turgid but the upper part had begun to shrivel. The tissues examined were full of bacilli. There was considerable variation in the breadth of the rods and some were much longer than others (samples were put into alcohol). The bundles were much broken down so that a large cavity had formed. None of the rods were motile. Five inches farther down (under the second leaf below the pricked one, *i.e.*, the leaf which had wilted the preceding day) the vessels of the bundles were gorged. The tissue here was but little broken down and the parenchyma was nearly free from bacilli. A small portion of the rods were actively motile. One and one-fourth inches farther down (in the hypocotyl) the bacteria were confined to the vessels and a portion of them were motile. The bacilli strung up from the cut stem 2 to 6 cm. when touched with a needle. Beef-broth-cultures were made December 24 from the interior of the stem where some of the bacilli were observed to be motile. Potato-cylinders inoculated from one of these broths (December 27) yielded in four days a rather scanty, wet-shining, white growth.

(97.) Extra early Hackensack cantaloupe (*Cucumis melo*). This plant was 3 inches high and had two small leaves besides the cotyledons, which were still green. Many pricks were made on the first true leaf, the blade of which was about 1.25 by 0.75 inch. The eighth day (9 a.m.) the pricked leaf was almost wholly flabby. It was normal at 2 p.m. the preceding day. The ninth day the vine was wilted and was brought into the laboratory and examined microscopically. Many bacilli were present in the vessels, but there were not so many as in 75 and 77, inoculated 4 days earlier, and examined the same day.

*Remarks.*—This series of inoculations, and that of December 6, settled the fact that the muskmelon disease is identical with that of the cucumber. I was in much doubt about the squashes. Only one plant (No. 79) had contracted the disease while all the squashes in this series and those in several other experiments (November 26 and December 6) refused to take the disease. I then interpreted these results as perhaps due to individual or varietal resistance on the part of the squash-plants experimented with since subsequent experiments performed in the same way gave positive results (see Nos. 215, 216, 217, 218, and 220).

No. 91 was very instructive in that it confirmed two suspicions: (1) The number of motile bacilli increases as one gets farther and farther away from the point of infection (*i. e.*, among younger rods); (2) The organisms pass down the vessels a long distance ahead of the signs. In this case the inoculated leaf was certainly removed within less than 22 hours and probably within 12 to 18 hours of the appearance of the first signs, while the greater part of the blade of the leaf (at least five-sixths of it) was still apparently sound and while the stem was still separated from the nearest wilted part by a distance of 5.5 cm. Nevertheless the bacilli had already passed down into the stem, so that the progress of the disease was but little if any slower than it would have been had the leaf not been cut away.

The culture used in making these inoculations had the following history:

- (1) Sept. 1. Typical diseased cucumber vine brought in from Anacostia, D. C.
- (2) Sept. 1. Vine No. 2, inoculated with white sticky slime direct from above plant. First sign the fourth day.
- (3) Sept. 17. Beef-broth-culture direct from the interior of vine 2.
- (4) Sept. 27. Slant meat extract peptone agar streak culture from 3.
- (5) Oct. 17. Potato-cylinder inoculated from one colony in No. 4.
- (6) Nov. 12. Potato-cylinder from 5.
- (7) Nov. 15. Potato cylinder from 6.
- (8) Nov. 20. Streak on a slant tube of unfiltered alkaline potato-agar.
- (9) Dec. 3. Potato-broth tube No. 2 from 8.
- (10) Dec. 6. Potato cylinder No. 5 made from 9.
- (11) Dec. 10. Plants inoculated from Tube 5, December 6, which now contained the same wet-shining, white, motile, and very sticky bacillus with which I started on September 1.

On December 20 the squashes were in 4-inch pots and growing satisfactorily.

#### INOCULATIONS OF JANUARY 3, 1895.

Potatoes, hyacinths, squashes, tomatoes, muskmelons, pear and cucumber were inoculated in the hothouse with a white, sticky schizomycete from a slant meat extract peptone agar culture of December 28 (cucumber-strain). The culture was examined the day the inoculations were made and found to consist of bacilli, a large proportion of which were motile. Great care was taken to avoid contamination, to use the bacteria as soon as taken from the surface of the agar, and to make the needle-punctures as small as possible. My method in this instance was to put a little of the white, sticky mass on the surface of the plant (leaf or stem) and then prick it in elsewhere, touching the needle tip to the slime each time before inserting it. Each plant received many punctures. In case of the squashes extra pains was taken to select full grown or nearly full grown leaves, and to make many very small needle-punctures, so as to prevent the bacteria from drying out and to secure their introduction into suitable tissues. Up to this time the inoculations into squashes had been unsuccessful. The temperature in the hothouse was 68° F. For the past month the day temperature had been about 70° F.

(98.) Potato (*Solanum tuberosum*). A very thrifty plant, growing in a 4-inch pot, was pricked on a terminal leaflet and in the middle part of the stem.

No result.

(99.) Potato. This plant was growing in a 4-inch pot, was 14 inches high and very thrifty. It was pricked on one end-leaflet and in the middle portion of the stem.

The eighteenth day the vine was examined for bacteria. The top had been shriveled for a week, and all the leaves had fallen. The rest of the stem, including the pricked parts, was normal. No bacteria were found in the vessels of the stem between the pricked part and the shriveled tip.

(100.) Hyacinth (*Hyacinthus orientalis*). The leaves of this plant were two inches long at the time of inoculation. The flower scape had not yet elongated but the buds were visible. It was a very healthy plant. Many pricks were made on the apical part of four different leaves.

No result.

(101.) Hubbard Squash (*Cucurbita* sp.). Many pricks were made on the apical portion of the blade of the sixth leaf of a small thrifty vine.

The twenty-fifth day the plant was brought into the laboratory for examination. Most of the leaves had fallen except those toward the apex where they were normal but small. The stem was green and long. It was examined in several places but not a trace of bacteria was found. The plant was crowded in a 4-inch pot.

(102.) Hubbard Squash. A comparatively large, thrifty plant was inoculated. Many pricks were made on the apical portion of the blade of the eleventh leaf. This leaf was about 6 inches from the apex of the vine.

The twenty-sixth day the plant was brought into the laboratory and examined. It was at this time 123 cm. long. Only the upper 30 cm. were leafy. The stem was green and turgid throughout. The stem was examined microscopically in three places—toward the base, in the middle, and toward the top. There was not a trace of bacteria. The plant had been kept in too small a pot.

(103.) Red Hyacinth (*Hyacinthus orientalis*). This plant bore leaves only about 2 inches long and the flower-stalk had not yet elongated. The plant was a very thrifty one. Many pricks were made on the apical portion of each of four leaves.

No result.



(104a.) Summer Squash (*Cucurbita* sp.). This vine was about 2 feet high at the time of inoculation. It was pricked on the apical part of the blade of a leaf toward the top of the vine.

The seventh day the pricked leaf had turned yellow and had become flabby on the pricked side but it was the lowest leaf and I could not tell whether the wilt was due to bacteria or to other causes. One day later the pricked leaf had wholly collapsed and shriveled. The other leaves were normal. Up to February 25 there had been no further wilt and no bacteria were found in the vessels. There was a small pocket on one side of the stem between the epidermis and sclerenchymatic ring in one place where a section was cut. This was filled with small bodies resembling bacteria but none were found in the deeper tissues. This plant was crowded in a 4-inch pot.

(104b.) Summer Squash. This vine was growing in the same pot as 104a. It was about 15 inches high and was pricked on the apical part of the blade of a leaf toward the tip of the vine.

The sixth day one side of the pricked leaf showed a faint trace of the wilt. The following morning half of the pricked leaf had wilted and had begun to shrivel and hang down. The blade of a small leaf 0.5 inch above had also collapsed. All the other leaves were normal. Twenty-five hours later both of the wilted leaves had shriveled. The rest were turgid. Six days later, the fourteenth day after inoculation, the rest of the leaves had wilted but the stem was still turgid. It was then brought into the laboratory and examined. Sections of the stem were cut in the vicinity of the pricked leaf (above and below) and also an inch farther up. There was not a trace of bacilli in the vessels or parenchyma. Many vessels were full of tyloses. The plant was erect and only about 15 inches high. It was growing in a small pot and had been overtopped and partly crowded out by a larger plant growing in the same pot. Some mildew was growing on it.

(105a.) Summer Squash. This vine, which was about 2 feet high and was in blossom, was pricked on a side lobe of a well-developed leaf. Many very small punctures were made.

On February 25 there was no wilt and no bacteria were found in the vessels or parenchyma.

(105b.) Summer Squash. A vine about 15 inches high, growing in the same pot as the preceding, was pricked on the apical portion of a well-developed leaf.

There was no wilt and an examination after 53 days showed no bacteria in the vessels or parenchyma.

(106.) Tomato (*Lycopersicon esculentum*). A thrifty but watery vine about 2 feet high, growing in a 4-inch pot, was pricked on one leaf and also very thoroughly the whole length of a middle internode including its two nodes. Thousands of living bacteria were put in.

No result.

(106a.) Check plant in the same pot.

(107a, b.) Tomato. Two very healthy but rather watery vines about 20 inches high were inoculated: one was pricked very thoroughly the whole length of a middle internode and its two nodes, the other was pricked on one leaf only.

No result.

(107c.) Check.

(108a.) Muskmelon (*Cucumis melo*). A small muskmelon vine was pricked on the blade of one leaf.

The eighth day there was a small wilted place on the apex of the pricked leaf, arising from the needle-stabs. Twenty-four hours later one-third of the apical portion of the pricked leaf had wilted. The tenth day all of the pricked leaf had wilted. January 31 the last leaf shriveled. The next day the plant was examined. There were no bacilli in the vessels of the stem and the barest trace of them in the petiole of the pricked leaf. None of the vessels were stopped up. Toward the end this plant was badly attacked by mildew, and its death must be ascribed to this fact and to the small pot, although it was infected by the bacteria.

(108b.) Muskmelon. This vine, which was small and was growing in the same pot as the preceding, was pricked on the blade of one leaf.

The morning of the sixth day the pricked leaf showed a wilt spot on the side of the blade near the margin. This was in the pricked area and included only about one-twentieth of the blade. Twenty-four hours later about one-third of the pricked leaf had wilted and the following morning three-fourths of the leaf-blade had become flabby and wilted. The petiole and the other leaves were turgid. The next morning the whole of the pricked blade had wilted and also that of the first leaf up. The petioles were still turgid. The next day (afternoon) the upper part of the stem had wilted. The vine was cut and put into alcohol for sections. Bacteria were demonstrated in the bundles of the stem by a microscopic examination.

(108c.) A check melon in the same pot remained free from the disease.

(109a.) Muskmelon. This was a small vine and was pricked on the blade of one of the leaves.

The fifth day three-fourths of the pricked leaf had wilted. The following morning the whole of the blade of the pricked leaf had collapsed. The eighth day the pricked blade was dry shriveling.

The petiole was still turgid and none of the other leaves were affected. Twenty-four hours later the tip of the petiole of the pricked leaf had begun to shrivel. The blade of the first leaf down had shriveled but the first leaf above showed no sign of the wilt in spite of the fact that the first internode below was three times as long as the first one above. The tenth day the first leaf up had collapsed.

(109b.) Muskmelon. A small vine in the same pot as the preceding was pricked on the blade of one leaf.

The morning of the fifth day one-eighth of the inoculated leaf (the apical pricked portion) had become dull-green and wilted. Twenty-four hours later the whole of the blade of the pricked leaf had collapsed. Two days later the pricked blade was dry shriveling. The petiole was still turgid and none of the other leaves showed any trace of the wilt. The following day half of the petiole of the pricked leaf had shriveled and the blade of the first leaf up had collapsed. The next day (afternoon) the upper part of the stem had wilted. The vine was cut and put into alcohol for study of location of bacilli by means of paraffin sections. They were found abundant in the vessels of the stem on microscopic examination.

(109c.) A small melon in the same pot as 109a and 109b was held as a check. It remained healthy.

(110a.) Muskmelon. A small vine was pricked on the blade of one leaf.

The fifth day seven-eighths of the pricked blade had wilted. Twenty-four hours later the whole of the leaf-blade had collapsed. Two days later the pricked blade was dry-shriveling, the petiole turgid. The blade of the first leaf down, which had begun to show signs of wilting the previous afternoon, had collapsed. The first leaf up was still normal. Twenty-four hours later the petiole of the pricked blade was flabby half-way down. The blade of the first leaf down had shriveled and that of the first one up showed very slight signs of loss of turgor. The first internode above was not half as long as the first one below. The next day the upper part of the stem was wilted and the vine was cut, brought into the laboratory and put into alcohol for sections: These when examined under the microscope showed the presence in the vessels of numerous bacteria.

(110b.) Muskmelon. The blade of the first true leaf of a small vine was pricked and inoculated.

The fifth day about one-fifteenth of the pricked leaf-blade had wilted in a small spot on one side near the apex and within the pricked area. Twenty-four hours later about three-fourths of the leaf-blade hung flabby. Two days later the pricked blade had begun to dry-shrivel. One cotyledon and the next leaf above the pricked one had begun to wilt. The following morning the petiole of the pricked leaf had begun to wilt at the apex. The blade of the first leaf up had wholly collapsed. January 13 the upper part of the stem was wilted and the vine was cut and put into alcohol. Sections examined under the microscope showed the presence of bacteria in the bundles of the stem.

(110c.) A vine growing in the same pot as 110a and b was held as a check and remained free from the wilt.

(111a.) Muskmelon. The blade of one of the leaves of a small vine was pricked and inoculated.

The fifth day over one-half of the pricked leaf blade (apical pricked part) had wilted. By the following morning the whole leaf had collapsed. Two days later the first leaf above the pricked one had begun to wilt. (Since 3 p.m. the preceding day). The morning of the ninth day the first leaf above had shriveled and the wilt had invaded the petiole of the pricked leaf. The plant was now removed and examined microscopically. The vessels were found to contain bacilli a part of which were motile.

(111b.) Muskmelon. A vine growing in the pot with 111a was pricked on one of its leaf-blades. The eighth day there was no trace of the wilt, but 24 hours later about one-fourth of the apex and one side of the pricked blade had wilted and changed to a dull green. The wilt began in the pricked area. The time from the insertion of the bacteria to the appearance of the wilt was about 8½ days, i. e., wilt appeared on the ninth day. The tenth day the stem was still turgid. Vine 111b was the last of the eight melons to show the disease. A microscopic examination was made and bacteria were demonstrated in the bundles of the stem.

(111c.) A vine growing in the pot with 111a and b was held as a check and remained free from the wilt.

(112.) Japanese Pear (*Pyrus* sp.). A small green shoot, 2 inches long, and a half-grown leaf of a Japanese pear seedling were pricked carefully.

The ninth day there was no trace of the disease, nor did any signs appear later on.

(113.) Cucumber (*Cucumis sativus*). An old cucumber vine growing in one of the insect cages (No. 38) in which infection had failed was pricked in the apical portion of one leaf-blade. The afternoon of the seventh day there were no signs of the wilt, but at 10 a.m. of the following day about one-sixth of the blade had wilted in the pricked area (fig. 66). The lowest sign of wilt was 1.5 cm. from the base of the blade, and the petiole was 5.5 cm. long. Thus there were 7 cm. of healthy looking tissue separating the diseased part from the stem. The leaf was now cut away close to the stem to

see if the disease could be prevented from entering the stem.\* Two days later the first leaf down had shriveled. The eleventh day the vine was brought in and examined in three places, *i. e.*, (1) at the base of the pricked leaf which was removed, (2) at the lower end of the same internode, (3) at the upper end of the next lower internode, *i. e.*, just below the last leaf to wilt. Not a trace of bacilli were found in the vessels or parenchyma, *i. e.*, the disease was cut out. This leaf (the last one on the vine) probably shriveled from the attacks of mildew, aphides and old age.

*Remarks.*—This virulent culture promptly attacked the eight muskmelons and the one cucumber, but had little or no effect on the squashes. It caused no disease in potatoes, tomatoes, hyacinths or pear. Theoretically it should have attacked the squashes readily and their behavior was a great puzzle. In five of the melons signs appeared on the same morning, *i. e.*,  $4\frac{3}{4}$  days from the insertion of the bacteria.

#### INOCULATIONS OF JANUARY 12, 1895.

Two squash-vines and one muskmelon-vine were inoculated with fluid taken directly from the interior of the inoculated wilted muskmelon vine No. 111a in which a microscopical examination had shown bacteria to be present. Many pricks were made with a small steel needle.

(114.) Summer Squash (*Cucurbita* sp.). Many tiny pricks were made on the blade of an upper leaf. On January 25, the pricked leaf-blade and two-thirds of the petiole were wilted (they were turgid the morning of January 24). This leaf was now removed and the petiole examined carefully, but I did not find any evidence of bacteria in the vessels or the parenchyma. The leaf was badly attacked by mildew.

February 25 there was no wilt, and on microscopic examination I could find no bacteria in the tissues of the stem.

(115.) Hubbard Squash (*Cucurbita* sp.). Many tiny pricks were made on the blade of one of the upper leaves.

February 27 there had been no wilt and three sets of sections from the stem at different heights showed no bacteria in the tissues.

(116.) Muskmelon (*Cucumis melo*). This vine which was a small one was pricked rather carelessly many times on the blade of a small leaf and held as a check on the squashes. The first wilt appeared the ninth day on the blade of the pricked leaf, involving about half of it. The following day the blade and petiole of the pricked leaf had begun to shrivel. The vine was brought in and its interior examined microscopically. The base of the petiole was full of the bacilli, a large proportion of which were very actively motile. The motions consisted of a straight ahead, sinuous, slow or rapid movement for long distances in all directions; a somewhat slow, curved movement carrying the rods long distances; and a slow or rapid tumbling motion.

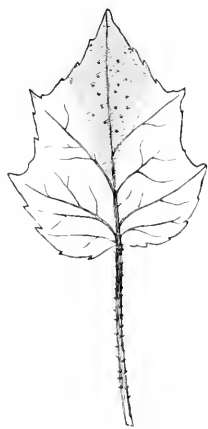


Fig. 66.†

*Remarks.*—Up to February 28, 1895, the experiments with squashes were very discouraging. The failure to induce the wilt with virulent bacteria, capable of wilting cucumbers and muskmelons in from 6 to 10 days when introduced simply by needle-pricks (as shown repeatedly by control inoculations) remained unexplained. Squash after squash was examined microscopically with great labor, but with exception of No. 79 and 104 a, no bacilli were found in the tissues. The inoculated plants were inspected day after day for many weeks, but none of them exhibited any distinct signs of secondary wilt, with the possible exception of No. 79. In case of a few plants inoculated late in the autumn a small wilted area appeared in the vicinity of the pricks, but did not increase much after its first appearance. In most of the squashes (36 were inoculated) no primary wilt appeared.

My conclusions at this time were that the squash-disease must be due to the organism causing the melon-wilt and cucumber-wilt.

\*The whole of the petiole (exclusive of a few sections from the base cut to examine microscopically) and the lower part of the midrib were put into alcohol along with the upper and partially wilted portion of the blade, for paraffin infiltration. It is important to determine how generally diffused in the parenchyma the bacteria are when the wilt and change of color (to pale green) occurs. Query: Does this depend on destruction of tissues or only on lack of water? (See fig. 68.)

†Fig. 66.—Leaf of inoculated plant No. 113 (cucumber) showing pricked area and extent of wilt on eighth day. There was none on seventh day. From basal part of wilted area to stem was a distance of 7 cm. (see text).

Up to this time the evidence in favor of the oneness of the squash-disease and cucumber-disease rested on the following facts:

(1) One case of the wilt disease in cucumber obtained by inoculating *four* plants with the milky bacterial ooze taken directly from the interior of a diseased squash-stem (September 1, 1894).

(2) Primary wilt on several squash-leaves inoculated November 17, 1894, with a pure culture obtained from a cucumber.

(3) The rather inconclusive evidence afforded by the presence of bacilli in some of the vessels of plant No. 79, examined  $2\frac{1}{2}$  months after inoculation, fungi being present also in some parts of the stem.

Opposed to this were many failures to convey the disease by needle-pricks using pure and virulent cultures of the bacillus. In my experiments on these squashes some unknown conditions necessary to infection were not fulfilled. In nature the squash takes the disease readily enough although it succumbs to it much less easily than the cucumber. That the disease is inoculable from squash to squash was also established by my experiments in Michigan in September, 1893. Whether the squash would contract the disease in the field as readily from melons and cucumbers as from squashes, remained to be determined.

#### INOCULATIONS OF MARCH 18, 1895.

In the hothouse 24 tomato-vines and 6 Japanese pear-seedlings were inoculated with bacteria from a white, wet-shining, thin, sticky, motile growth on slant agar (tube No. 3, March 13, from stab No. 4, January 9. The culture in great part was much younger than March 13, the uninoculated surface of the slant having been spread over with bacteria from the other parts on March 16). Each plant was given a dozen or more pricks. The bacteria were lifted on a sterilized cooled needle. The inoculations were begun about 10 a. m. and finished at 4.30 p. m. It was a windy drying day with a hot sun under the glass. The tube was carefully shielded from all but very short exposures to direct sunlight. Thousands of living bacilli were pricked into each plant. The needle and loop were flamed before each set of inoculations and not used till cool. As an additional precaution the needle was always thrust first once or twice into the cool stem and then used. A loop of the white slime was put on the surface of the stem and stabbed in repeatedly. An examination of each one of the inoculated plants was made on March 27, April 15, May 9, and later dates.

(117.) Tomato (*Lycopersicum esculentum*). A thrifty vine about 30 inches high was pricked at the base of one of the upper branches (two nodes and the internode). The tissue was rather firm. There was no result (May 9).

(118.) Tomato (same plant). Two internodes and one node near the tender apex of a basal shoot. The tissues were soft and the needle entered very easily.

There was no result. By May 9 the shoot had grown 2 feet since it was pricked.

(119.) Tomato. A thrifty vine about 25 inches high was pricked in a node and two internodes in the apical part of a branch. The tissues were immature and tender.

Between the time of inoculation and May 9 the pricked branch grew over 18 inches. There was no result from the introduction of the bacteria.

(120 to 140.) Tomatoes of the same size and inoculated in the same way as the preceding.

No disease resulted. The 21 plants were under observation for 52 days.

(141.) Japan Pear Seedling (*Pirus* sp.). Pricks were made in the growing tip of a shoot.

There was no result.

(142 to 146.) Five Japan Pear Seedlings. Like No. 141.

No result.

*Remarks.*—None of the tomato-shoots or pear-shoots showed anything but slight local disturbances as a result of the inoculations. The pear-shoots blackened around the needle-punctures almost immediately (host reaction) and after that showed no change. The tomatoes swelled slightly around the pricks in some cases, and in others there was a slight falling in and discoloration of tissue immediately surrounding the needle-pricks, nothing more.

Clearly, therefore, Dr. Halsted's inoculations were not made with this organism. On April 5, five of the tomato-plants were watered as follows: Two with 500 cc. 0.1 per cent watery solution of  $\text{KNO}_3$ , one with 500 cc. of 0.1 per cent watery solution of kainit, one with 500 cc. 0.1 per cent watery solution of muriate of potash, and one with 500 cc. of water allowed to stand, with shaking on 2 grams of fine, dissolved bone ash. Five days later each plant was given another 500 cc. of the solution. There was no visible effect from the previous waterings. Only the nitrate of potash seemed to show a doubtful trace on one or two plants. There was nothing distinct and there was no change up to June 22.

For the check-plants see the next series.

#### INOCULATIONS OF MARCH 19, 1895.

Three cucumber-vines (*Cucumis sativus*) bearing 2 and 3 leaves were inoculated as checks on the tomatoes and pears pricked on March 18. The remnant of the slant agar-culture No. 3, March 13, was used for making the inoculations. A small, sharp, steel needle was used to introduce the bacteria.

(147.) Many pricks were made on one leaf. The third day, the vine rotted off at the base: It had been kept too wet and was attacked by nematodes.

(148.) Many pricks were made on one of the leaves. The sixth day at 9 a.m. there was no wilt but at 10:30 a.m. there was the slightest trace of wilt at the apex of the inoculated leaf. There was no change of color. The following morning half of the leaf-blade had wilted with a characteristic change of color. Twenty-four hours later (eighth day) the whole of the pricked blade had wilted except a small area near its base. The petiole was rigid. The tenth day there was a slight wilt of the first leaf up and the first one down. The following morning the two leaves last mentioned showed a decided wilt, the first one down being the more badly affected. The thirteenth day all the leaves were wilted and the vine, which was a small one, was cut for examination. The vessels of the stem were plugged by bacilli which were present in enormous numbers. They were also abundant in the midrib and smaller veins of the partially developed second leaf above the pricked one. No motile rods were observed in any of the sections, not even in those near the tip of the vine (specimens saved in alcohol).

(149.) Many pricks were made on one leaf. The fifth day (3 p.m.) there was no trace of the disease but the next morning the pricked leaf showed wilt at the apex. About one-fifth of the leaf was affected. There was yet no change of color. Twenty-four hours later over half of the leaf-blade was wilted with the characteristic change of color. The eighth day the whole of the pricked blade had wilted with the exception of a small area near its base. The petiole was turgid. The following day the first leaf on either side of the pricked one was slightly wilted. The eleventh day the blades of these two leaves showed decided wilt, that of the lower one being the most wilted. The thirteenth day all the leaves were wilted and the vine was cut for examination under the microscope. The vessels of the stem were plugged by enormous numbers of bacilli even to the extreme tip (fig. 67).

*Remarks.*—This sufficiently settled the virulent nature of the bacteria pricked into the pear-stems and tomato shoots out of this same pure culture the day before. We may, therefore, conclude that pears and tomatoes are not affected by this organism. The microscopic examination shows that bacteria are sometimes present in the veins of the leaf very soon after it wilts, whether they are always present in advance of the wilt or whether the secondary wilt is sometimes due entirely to occlusions of vessels in the stem or petiole remains to be determined (see fig. 68).

#### INOCULATIONS OF APRIL 15, 1895.

Muskmelons, cucumbers, squashes, pumpkins, watermelons and tomatoes were inoculated in the hothouse at midday with bacilli (cucumber-strain) from a pure, motile, slant agar-culture 48 hours old (tube 1, April 13, from agar stab No. 2, April 8). All the inoculations were made by needle-pricks. The loop and needle were flamed and cooled each time before using.

A fresh mass of the slime was fished out of the tube for the inoculation of each plant and the bacteria were pricked in immediately to avoid danger from drying.

The inoculations were made under a newspaper-shade to keep off the direct rays of the sun. The temperature was about 80° F. The vines which were planted March 12 were in good condition and had several leaves besides the cotyledons.

(150.) Muskmelon var. Shumway's giant (*Cucumis melo*). Many pricks were made in the middle and upper part of a leaf 2.25 inches broad. The morning of the fourth day there was no trace of the disease, but at 2 p.m. there was a distinct wilt covering about 1 sq. cm. near the apex of the pricked leaf. It was then 4 days and 3 hours since the leaf was pricked. Twenty-four hours later the



Fig. 67.\*

apical one-third of the leaf-blade had wilted and changed to a dull green verging on slightly yellowish (at 9 a.m. the wilted area was not much greater than on the preceding day). The following day (3 p.m.) two-thirds of the pricked leaf had wilted. The blade of the first leaf below was also flabby. The internode between these two leaves was very short, i.e., not over 2 mm. The temperature in the hothouse was over 90° F. The seventh day the blade of the first leaf above the pricked one had wilted. It was separated from the latter by an internode only 2 cm. long. The second leaf below, a

\*FIG. 67. Cross-section of extreme top of cucumber-vine No. 149 some days after infection with *B. tracheiphilus*. The plant was inoculated on the blade of a leaf by needle-pricks. The bacteria are a long distance from point of inoculation and confined strictly to the bundles, all of which are invaded. Drawn from slide No. 205-2.

green cotyledon separated from the pricked leaf by a distance of 2 cm., was also wilted. At 4 p.m. of the same day the vine was brought into the laboratory and photographed along with a healthy vine of the same age (Vol. I, fig. 8). The first leaf below the pricked one was examined for bacilli in the veins of the blade. They were present but not yet numerous. The leaf-blade had been wilted rather more than 25 hours (samples were put into alcohol for the microtome). The blade of the first leaf up which was normal at 3 p. m. the previous day but had wilted either the morning of the seventh day or the previous night, was also examined: The spiral vessels at the apex of the petiole contained numerous bacilli. The veins of the blade were not examined but samples were put into alcohol for sections. Bacteria were also found in the vessels of the uppermost small leaf at the junction of blade and petiole. The petioles of these leaves (pricked one included) were rigid and neither these nor the stem had changed color. For the appearance of a single infected bundle in cross-section see fig. 69. For appearance of a whole petiole in cross-section with low magnification consult Vol. I, pl. 3.

(151.) Cucumber var. White wonder (*Cucumis sativus*). Many pricks were made in a basal lobe of a leaf about 2.25 inches across. The morning of the fifth day the first slight trace of the wilt appeared in the pricked part of the leaf. At 2 p. m. the wilted part was duller green and covered an area of nearly 1 sq. cm. in the middle of the pricked part. The following day (3 p. m.) there was only a small increase of the wilt: Not one-twentieth of the leaf-blade was involved. The eighth day about one-fourth of the pricked leaf was flabby and the middle of the diseased area was now brown and dry. The following day the pricked side of the leaf was dry-shriveled and the rest of the blade had wilted. The petiole and the other leaves were turgid. The ninth day the first leaf down (3 cm. below) wilted and the following day the first leaf up (2 cm.) drooped its blade. The second leaf up was then turgid (9 a. m.), but at 1 p. m. its blade was drooping. The petioles were still rigid except that of the first leaf down which was a very slender one. The fifteenth day after inoculation all the leaf-blades were wilted but the stem and all the petioles were green and turgid. Thin sections of the blade of the second leaf up were now examined under the microscope and the bacilli were found to be plentiful in the spirals of the leaf-blade and forming cavities around them. They were apparently not in the green parenchyma-cells. Portions of the petiole of the pricked leaf were put for 2 minutes into boiling absolute alcohol containing 1 per cent picric acid and were then transferred to 75 per cent alcohol which was repeatedly renewed, *i. e.*, until all the picric acid was removed. Other portions were fixed directly in 75 per cent alcohol. The first mentioned fixative gave the best results. The petiole of the pricked leaf was also examined microscopically. The vessels were gorged with bacilli and the primary vessel-parenchyma was broken down.

(152.) Winter Squash var. Sibley's or Pikes Peak (*Cucurbita* sp.). Many pricks were made on a leaf 3 inches broad, on one side about midway from the base to the apex of the blade. The ninth day there was no trace of the wilt, nor did it appear later.

(153.) Winter Squash var. Sibley's or Pikes Peak. Many pricks were made on the apical part of a blade about 2 inches broad. There was no result from the inoculation.

(154.) Winter Squash (same variety). Many pricks were made on one side of the blade of a leaf about 2.5 inches broad.

No result.

(155.) Winter Squash (same variety). This was growing in the same pot as 154. Many pricks were made on the apex of the blade of a leaf about 2.25 inches broad.

There was no result from the inoculation.



Fig. 68.\*

\*FIG. 68.—B, cross-section of a squash-leaf wilted by *Bacillus tracheiphilus*, showing that wilt of parenchyma is due to cutting off water-supply rather than to actual occupation of parenchymatic tissues by the bacteria. At base is a bundle destroyed by the bacteria. Beyond this is a long wilted area in which no bacteria occur. Only a portion of this wilted area could be shown in the picture, the whole length being shown in fig. C; the portion represented in the drawing corresponds to the black part of C. A, neighboring uncollapsed portion of the same leaf, the bacteria in this being confined to a portion only of the vessels of the bundle. Slide 362-1, lower row, last section but one at the right. Drawn with a Zeiss 8 mm. apochromatic objective, No. 12 eye-piece, and Abbe camera.

(156.) Pumpkin var. Nantucket Sugar (*Cucurbita* sp.). Many pricks were made on various parts of a leaf-blade about 2.75 inches broad.

There was no result from the inoculation.

(157.) Pumpkin var. Nantucket Sugar. This vine which was growing in the same pot as the preceding was pricked many times on the middle and apical portion of a leaf-blade about 3.5 inches broad.

There was no result from the inoculation.

(158.) Pumpkin var. Nantucket Sugar. This vine was in the pot with 156 and 157. Many pricks were made on the middle and apical part of a blade about 2.5 inches broad.

There was no result from the inoculation.

(159.) Pumpkin var. Nantucket Sugar. Many pricks were made on the apical part of a leaf-blade about 2 inches broad.

There was no result from the inoculation.

(160.) Pumpkin var. Nantucket Sugar. This was growing in the pot with the preceding. Many pricks were made on the apical portion of a leaf-blade about 2 inches broad.

No disease resulted.

(161.) Pumpkin var. Nantucket Sugar. Many pricks were made on the apical portion of a leaf-blade about 3 inches broad.

No result.

(162.) Pumpkin var. Nantucket Sugar. This vine was growing in the pot with 161. Many pricks were made on one side of a leaf-blade about 4 inches broad.

No result.

(163.) Watermelon (*Citrullus vulgaris*). This vine, which was planted March 12, was pricked many times on a side lobe of a leaf-blade about 1.75 inches broad.

There was no result from the inoculation.

(164.) Watermelon. This vine was about a month old. Many pricks were made on the apical part of a leaf-blade about 1 inch broad.

No result.

(165.) Watermelon. This vine was the same age as the preceding. Many pricks were made on the middle lobe on one side of a leaf-blade about 1.25 inches in diameter.

No result.

(166.) Watermelon. This was growing in the pot with the preceding. Many pricks were made on the middle and basal lobes on one side of a leaf-blade about 2 inches broad.

No result.

(167.) Muskmelon var. Shamway's Giant. About 20 pricks were made in the center of a leaf-blade over 2 inches broad. The pricks were in a space not over 5 mm. in diameter, and to each side of the midrib. The fifth day (9 a. m.) the first trace of wilt appeared. It extended in a narrow line along the midrib from the pricked area to the tip. It was most noticeable at the extreme tip. At 2 p. m. the apical one-sixth of the leaf-blade had wilted in a V-shaped area from the pricked part outward. The wilt did not yet extend downward beyond the pricked area more than 1.5 mm. The next afternoon about one-third of the pricked leaf-blade (apical part) had wilted and changed color. The seventh day there was no change. The next morning the blade of the first leaf down and of the first leaf up had wilted. The petioles were rigid. About two-thirds of the blade of the pricked leaf was flabby. The ninth day the cotyledon under the pricked leaf had wilted. The opposite one was green and turgid. The second leaf up was flabby. The leaves of the bud were still normal although

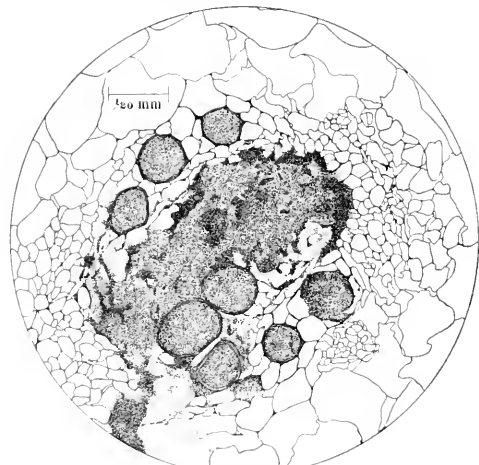


Fig. 69.\*

\*FIG. 69. — Cross-section of middle of a muskmelon petiole, showing a bundle disorganized by *Bacillus tracheiphilus* with the formation of a large cavity. From inoculated plant No. 150. Drawn from a paraffin infiltrated stained section. Slide No. 208 A 9.



they were lower down than the blade of the second leaf which was wilted. The tenth day the petioles were still rigid but all the blades were drooping except of one cotyledon and on the tiny leaves of the bud which were not transpiring much. The fourteenth day all the leaves were wilted. The petioles and stem were still bright green and turgid.

The plant was now examined microscopically. The vessels were gorged with the bacillus and there was an extensive degeneration of the spiral vessels and of the primary vessel-parenchyma (slide 210). Portions of this vine were preserved in three different ways to determine the best way to fix the bacilli and slime in the vessels without shrinkage of the tissues of the host: (1) The first lot was put directly into absolute alcohol which began to remove the chlorophyll inside of two hours so that the lower half of the fluid was decidedly green; (2) 75 per cent alcohol which fixed the slime and in two hours had not withdrawn any chlorophyll; (3) 50 per cent alcohol which did not fix and was worthless. In the 50 per cent alcohol the bacilli oozed out within 2 hours in quantity and formed a milky slime in the bottom of the bottle and all over the ends of the segments. There was not much choice between the effect of absolute alcohol and 75 per cent alcohol.

(168.) Cucumber var. White Wonder. About 20 pricks were made in the center of a leaf-blade about 2.5 inches broad. The pricks were all in an area not over 5 mm. in diameter and each side of the midrib. The eighth day there was no trace of the disease but the following day (11 a.m.) the wilt appeared over an area  $5 \times 15$  mm. extending from the pricked part outward toward the tip along a side vein. The tenth day there was still no wilt except on the pricked leaf. The narrow oblong area which appeared wilted the day before had now dried out and much fresh tissue was involved in the wilt, about one-sixth of the whole leaf being affected, *i. e.*, each side of the midrib to the apex and also downward over half-way to the base of the blade. The seventeenth day the vine was brought into the laboratory and dissected. The leaf-blades were all wilted some days before. With the exception of the lowest petiole which was wilted and somewhat yellow at the tip, the petioles were all green and turgid. The stem was turgid and normal in external appearance. There was no rot at the base of the plant. The interior of the vine was full of bacilli.

No. 168 was the fourth and last check against the squash and pumpkin inoculations, none of which had given any positive results. The internodes of this vine were cut into short lengths and put alternately into two bottles. Those in one were covered for 22 hours with 1 per cent tri-nitro-phenol dissolved in absolute alcohol; the others were put for the same time into absolute alcohol saturated with mercuric chloride. The former proved the best fixative.

(169.) Tomato (*Lycopersicon esculentum*). About 20 pricks were made in a green fruit about one inch in diameter. Some of the pricks were shallow and some were deep. Many thousands of the bacteria were put in. The fourth day the fruit was one-third larger. There was a very narrow rim of dead tissue about the pricks and beyond this a narrow ring of tissue which was darker green than natural. This second ring was not over 0.4 mm. broad. On May 9, 24 days after inoculation, the tissue around the pricks was slightly sunken. The immediate border, 0.2 mm. in width, was dead; beyond this for a short distance (0.5 to 1 mm.) the tissue was a little darker green than natural. There were no other signs. The fruit had become three times as big as when pricked and the other fruit on the cluster (earlier set) was ripe. On May 14 the inoculated fruit was ripe. The second ring referred to above, ripened more slowly than the rest and was still greenish. The fruit was sound, normal and well flavored.

(170.) Tomato. A green fruit about an inch in diameter was pricked 12 times and a great many bacteria were inserted. There was no result from the inoculation. The fourth day it resembled the preceding. On May 9 the four fruits in the cluster were all growing finely. The pricked one was exactly like 169 at this date. On May 20 it was fully ripe and was picked and eaten. It was entirely sound. The only result from the pricks was death of ruptured cells and retarded ripening just around the track of the needle.

*Remarks.*—The following note of June 10, 1895, may be of interest in connection with the observations on Nos. 169 and 170.

Two small green tomato fruits pricked with a pin some weeks ago have become darker green around the pricks just as did those previously inoculated with *Bacillus trachicophilus*. Apparently the phenomenon is the reaction of the plant against the puncture and not against the bacteria.

This experiment confirmed the earlier ones. Cucumber and muskmelon were found susceptible to the culture used while squash, pumpkin and watermelon were resistant. All of the former and none of the latter contracted the disease.

## INOCULATIONS OF MAY 13, 1895.

Nineteen potato-vines were inoculated in the hothouse with *B. tracheiphilus* (cucumber-strain) the virulence of which was checked by inoculation into five cucumbers. The potatoes were planted in 7-inch pots April 23. Large tubers were halved and all but two eyes cut out. At the time of inoculation each pot bore five or six shoots 8 to 10 inches high, growing rapidly and all very thrifty. The cucumbers (*Cucumis sativus*) were several months old, 2 to 3 feet high and in bloom. The bacteria used for inoculation were from a pure slant agar-culture (tube 1, May 11) made for this purpose from a glycerin-agar-culture (No. 8, May 1, reinoculated May 8). The growth was a characteristic, smooth, wet-shining, milk-white streak, much more sticky than the glycerine-agar-culture from which it was made. The weather was cool. The temperature in the hothouse at the time of inoculation was 80° F. All the pricks made were deep. The needle and loop were flamed and cooled each time and thousands of living bacteria were thrust in. The potatoes received many pricks into young leaves and tender shoots.

(171 to 189.) Potato (*Solanum tuberosum*). No result.

(190.) Cucumber. The inoculation was made in a leaf borne on the tenth node (there were many nodes above). Many pricks were made near the midrib about half-way from the base of the blade to the tip. The blade was about 5 inches broad. Up to 1 p.m. May 27 (end of the fourteenth day) there was no trace of the disease, but at 5 p.m. of that day a very small area (less than one square centimeter) was wilted. The eighteenth day no constitutional signs had appeared—only wilt and shriveling of the pricked leaf-blade. Half of the latter was dry-shriveled and the rest hung flabby. There was no wilt above or below this leaf. Twenty-one days after the inoculation the pricked blade was wholly dry-shriveled and of a brownish color. The petiole was green and turgid except the upper inch which had become slightly yellowish and a little flabby. The leaves above and below were normal. Three days later there was still no wilt of the leaves above or below. The tip of the petiole of the pricked leaf was flabby. On the beginning of the twenty-fifth day the first secondary wilt appeared. This was in the first two leaves above the pricked one. The rest of the leaves were turgid. The general infection of the plant was very slow. June 8th (26 days after inoculation) the blade of the first leaf below the pricked one showed wilt (9 a.m.). At noon the blade of the third leaf up and of the second leaf down were wilted and that of the first leaf up had dry-shriveled the same as the pricked leaf. June 15 the vine had lost all its leaves by the wilt but the stem was yet green and turgid. A petiole was now cut across and the sticky bacterial ooze was pricked into the leaves and stems of pumpkin and squash. (No. 198 and others.) The same day the vine was cut and put into 75 per cent alcohol for microtome sections. It was not then examined microscopically but has been since—enormous numbers of bacteria being found in the vascular bundles of the stem.

(191.) Cucumber. Many pricks were made in the middle of a leaf-blade (5 inches broad) to one side of the midrib. The pricked leaf was on the tenth node. The sixth day (2.30 p.m.) the leaf had wilted over an area of 1×3 cm. from the pricks outward, along both sides of a main vein nearly to the margin of the leaf. There was no wilt the preceding day at 4 p.m. The seventh day there was little change. Twenty-five hours later the bulk of the leaf was still turgid. The ninth day the whole leaf-blade drooped and the pricked side was drying out. Two days later the whole blade of the pricked leaf had shriveled. The petiole was still green and rigid. In the afternoon of the eleventh day the blade of the first leaf up began to droop decidedly on one side. The following morning it had partly recovered its turgor. At 2 p.m. the leaf-blade hung down flabby. The fourteenth day the blades of the first, second and third leaves up had collapsed and also those of the first and second down. The petiole of the pricked leaf was beginning to shrivel in the upper two inches. Four days later the leaf-blades were all down. The petiole of the pricked leaf had shriveled nearly to the base. The petioles below were turgid but those above were beginning to be flabby.

(192.) Cucumber. Many pricks were made in the middle apical part of a leaf-blade about 4.5 inches broad. The leaf was on the eleventh node. The sixth day (2<sup>h</sup> 30<sup>m</sup> p.m.) the leaf had wilted from the pricked part to the apex, a length of 4 cm. and a breadth of about 1 cm. The following morning there was little change. The eighth day the bulk of the leaf was still turgid. The day was cool, cloudy, and rainy. The following day about half of the blade of the pricked leaf had wilted. The petiole was rigid. The eleventh day the whole blade of the pricked leaf had shriveled. The petiole was still green and rigid. Twenty-four hours later the petiole of the leaf was still normal externally as was also the first leaf to either side of the pricked one. Four hours later the blade of the first leaf up had wilted. The fourteenth day the blades of the first and second leaves down collapsed.

The petiole of the first leaf down was rigid and green. That of the second leaf down was flabby and drooping. It was a smaller petiole. The blades of the first, second, third and fourth leaves up had collapsed. The petiole of the pricked leaf was still normal. The eighteenth day all the leaf-blades had collapsed except the two lowest. The petiole of the pricked leaf was still green and turgid as was the case with all those below it. All the petioles above the pricked leaf were flabby, especially toward their tips.

(193.) Cucumber. Many pricks were made on the middle apical portion of a leaf-blade 5 inches broad. The leaf was on the ninth node. The 6th day (2 p.m.) the leaf was wilted from the pricked place to the apex. The wilted area widened outward, being about 1 cm. broad at the pricks and 2 to 3 cm. wide farther up. Two days later the bulk of the leaf was still normal but the wilt was spreading slowly. May 22, 9 days after inoculation, the whole blade of the pricked leaf had wilted. It was still green and the petiole was turgid. Two days later the whole blade of the pricked leaf had shriveled. The petiole was still green and rigid. The twelfth day (10 a.m.) the petiole of the pricked leaf was normal as was also the first leaf up and the first leaf down. Four hours later two-thirds of the blade of the first leaf up had wilted. The fourteenth day the blades of the first and second leaf down were flabby. The petiole of the first leaf down was rigid. The smaller petiole of the second down was flabby and drooping. The blades of the first and second leaf up were wholly flabby and the third up was beginning to show signs of the wilt. The petiole of the pricked leaf was still normal. Four days later the blades of the first three leaves below were dry-shriveled and the petiole of the pricked leaf was flabby at its tip. The petioles of the first and third leaves down were turgid and green. That of the second leaf down was shriveling at its apex. The blades of the first four leaves up were shriveled and the petioles flabby. The blades of the next two above were flabby but the rest of the leaves (half a dozen still farther up) were normal.

(194.) Cucumber. Many pricks were made in the middle of the blade of a small leaf. The ninth day there were no signs but 2 days later the pricked leaf-blade had a narrow, shriveled, dry strip extending from the pricks to the tip ( $0.8 \times 3$  cm.) showing that the wilt had appeared the preceding day. The twelfth day the whole blade of the pricked leaf had shriveled. The petiole was turgid as were also the leaves above and below. The fourteenth day the first leaf down shriveled. Four days later all the leaves were down and the stem was bowed over in the middle. This was a small vine.

*Remarks.*—Three of the cucumbers came down on May 19 (sixth day), and Nos. 191 to 194 were very badly diseased before No. 190 showed any constitutional signs. Signs outside of the inoculated leaf did not appear on the latter until after 24 days. From that time on the progress of the disease was as usual.

May 29, and 30 were very hot.

On May 21, all of the potatoes were healthy. There was some tearing of the pricked tissues due to rapid growth and on some of the stems there was a superficial blackening of the pricks but no disease resulted. On June 30, all of the potato plants were still free from the disease.

These numerous inoculations on potato and tomato were made owing to statements by Dr. Halsted (in Bull. 19, Mississippi Agric. Exp. Station, and elsewhere) connecting causally the southern bacterial tomato blight with a bacterial rot of melons observed by him in Mississippi, New Jersey, and elsewhere, and confused at that time, at least in my own mind, with this disease.

#### INOCULATIONS OF MAY 25, 1895.

A series of inoculations was made at 3 p. m., on cucumbers (*Cucumis sativus*) by spraying the striped cucumber-beetle, *Diabrotica vittata*, with dilute broth containing *Bacillus tracheiphilus* and placing them on the plants in an insect cage. The culture used was one in slightly acid potato-broth (tube 3, May 22), containing rolling clouds when shaken. It was examined in a hanging drop and found to contain comparatively few rods, some of which were feebly motile. It was diluted with three times as much distilled water before using for the cage-experiments.

(195 a to f.) Cucumbers. Six small, old, rather stunted cucumber-vines, from which most of the aphides had been removed, but which would never amount to much without repotting (in 4 pots) were placed in an insect cage. The soil outside and in was thoroughly wet down and a dozen or two

specimens of *Diabrotica vittata* were turned loose on them after the insects had been thoroughly sprayed with the dilute broth and left to crawl about in the infected liquid half an hour. Many of the leaves were already whitish on the margins and there were brown spots on the others and some mildew (*Erysiphe chicoracearum*). July 16. The experiment failed.

(196 a to g.) Cucumbers. Three pots containing seven plants much like those just described were placed in an insect-cage. The lower leaves were injured more than the preceding and there were large dead patches on the margins of some of the leaves and others were becoming whitish probably from malnutrition. The soil outside and in was thoroughly wet down and the wire of the cage was also wet so as to keep the air inside moist for the next 24 hours. The surface soil of the pots was then slightly sprayed with the dilute broth and also, thoroughly, every part of each plant—stems, young fruits, open flowers, old and young leaves (both sides), and the buds and leaf axils, so to as cause the disease if possible. The infectious material was from the same tube as that used for 195. Almost all the aphides were removed but not quite all. The broth used for infection was not very satisfactory. It was not swarming with rods, *i. e.*, there was only here and there one in the hanging drop although of course where so much fluid was sprayed on, the aggregate number of rods was large. The inoculations were made on a cloudy, cool, rather damp afternoon. The plants in this cage were to be held as a check on the preceding. July 16. There was no result from the inoculation.

#### INOCULATIONS OF JUNE 15, 1895.

Squash-vines (*Cucurbita* sp.) and pumpkin-vines (*Cucurbita pepo*) were pricked and inoculated at noon with sticky bacterial ooze directly from the interior of a petiole of the inoculated cucumber-vine 190. Two cucumbers (*Cucumis sativus*) were inoculated as checks. The day was sunny and hot. There is no statement as to the number of needle-punctures, but only that the checks were inoculated in the same way as the others.



Fig. 70.\*

(197.) Cucumber. This vine was inoculated as a check. No record of where inoculated but undoubtedly on some leaf-blade. The fourteenth day (noon) half of the foliage had collapsed. Two days later the vine was brought into the laboratory and examined. From the cut end of the stem there oozed a sticky white bacterial slime, drawing out in slender threads, and a microscopic examination showed that the vessels contained great numbers of a bacillus morphologically like *B. tracheiphilus*.

(198.) Squash. Two terminal small leaves were pricked. No result.

(199.) Cucumber. This vine was also inoculated as a check. On July 9 some of the leaves were wilted. It was brought in and examined microscopically. The vessels were found to be full of bacilli of variable size (fig. 70.)

(200.) Squash. The third leaf from the tip was pricked and inoculated. No result.

(201.) Pumpkin. The stem was pricked.

No result.

(202.) Pumpkin. The stem was pricked.

No result.

(203.) Squash. The stem was pricked.

No result.

(204.) Pumpkin. A leaf was inoculated.

No result.

*Remarks.*—The squash-plants and pumpkin-plants were kept under observation for 38 days. The squashes were grown from seeds planted March 12. Here again cucumbers contracted the disease while squashes and pumpkins resisted it.

\*Fig. 70.—Bacteria, especially aberrant forms, from interior of cucumber-vine No. 199, inoculated with *B. tracheiphilus*. The common forms are 1.8 to 2.5 by 0.6 to 0.7  $\mu$ . About 1 : 1000 or 1 : 2000 is much larger, but transition forms were observed. Rarely one with a distinct capsule was seen. Cover-glass preparation stained by van Emeric's nitrate of silver method. July, 1895.

INOCULATIONS OF OCTOBER 5, 1895.

A new set of inoculations was made in the hothouse, at 3 p. m., on young cucumbers (*Cucumis sativus*), gherkins (*Cucumis anguria*), young muskmelons (*Cucumis melo*), and young squashes (*Cucurbita* sp.), using sticky bacterial ooze from the interior of a cucumber-stem obtained from a field of late cucumbers a few miles northwest of Washington. The bacteria were very sticky and strung out a long distance from the cut end of the stem. The stem was examined microscopically and found to contain many vessels gorged with a bacillus. I washed the surface of the stem, then shortened it several times with a razor, and finally with a flamed needle pricked the oozing bacteria into the healthy plants. The wet sticky surface of the stem was also pressed down on the surface of the leaves and many delicate needle-pricks were made within the wetted area. Especial pains were taken in each case to make the infection thorough. The plants were 6 to 8 inches high except the gherkins which were smaller. All the inoculations were made on the leaf-blades.

(205.) Cucumber. The ninth day the pricked leaf had changed color and was drooping but the petiole was rigid. The blade of the next leaf up also drooped some but was a healthy green. Twenty-four hours later the blades of two more leaves were wilted but the petioles were still rigid. The cotyledons were not yet wilted although on nearly the same level as the base of the petiole of the pricked leaf. The eleventh day the cotyledons were drooping but all the petioles were rigid as also 24 hours later. The fourteenth day the vine was brought into the laboratory and examined. All the foliage had wilted and the stem near the earth had bowed over. Otherwise it was normal in external appearance. The upper part of the stem was cut and examined microscopically. The juice was sticky and stringy and the vessels were full of a bacillus which had also flooded out into the surrounding parenchyma and was motile. The rods were all nearly the same size. The stem was cut cross-wise with a hot knife and dug into with a hot needle and from the cavity thus made bacteria were removed for eight cultures: No. 1, old alkaline potato broth; No. 2, streak on alkaline agar; Nos. 3 to 8 potato cylinders. The agar failed; all the potato-tubes developed typical cultures of *B. tracheiphilus* of which four were exceedingly sticky, one moderately sticky, and one only slightly sticky (observations after 5 days).

(206.) Cucumber. The ninth day half of the pricked leaf-blade was drooping. The rest of the plant was normal but 24 hours later the blade of the first leaf up had wilted. The cotyledons which arose from nearly the same level as the pricked leaf showed no sign of the wilt. The petioles were still rigid. The following day the blade of the next leaf up had wilted. The cotyledons and all the petioles were rigid. Twenty-four hours later one of the cotyledons was drooping. The blades of the wilted leaves were badly collapsed but the petioles were still turgid. Four days later (the sixteenth day after inoculation) all the leaf-blades had shriveled and also the apex of the petioles. The twenty-third day the whole plant had shriveled.

(207.) Cucumber. By the end of the ninth day the entire blade of the pricked leaf had changed color and wilted. The blade of the first leaf up was also drooping but was of a good green color. Twenty-four hours later one of the cotyledons hung limp. The petioles were rigid. The following day the second leaf up began to roll at the edges. The twelfth day after inoculation the second cotyledon was drooping. The petioles of the wilted leaves were still rigid but the blades were badly collapsed. Four days later the blades and tip of the petiole were shriveled. The twenty-third day the whole plant was shriveled.

(208.) Muskmelon. The ninth day after inoculation the pricked blade changed color and showed a trace of wilt centering in a group of pricks. The total affected area was only a few square millimeters. The following day there was very little increase of the wilt, scarcely 2 sq. mm. Twenty-four hours later about one-third of the blade of the pricked leaf was plainly wilted. The following day more than two-thirds of the pricked blade had changed color and was drooping. The petiole was rigid. Four days later the blade of the pricked leaf had shriveled but the petiole was turgid. The next two leaves above now had wilted blades. A week later the whole plant had shriveled.

(209.) Muskmelon. The ninth day the tissue in one small group of pricks was dead. Three days later there was a small amount of wilt near the margin. The twenty-third day the pricked parts were dead and the tissue around them was yellow. The tip of the leaf had wilted slightly. The twenty-sixth day the entire pricked leaf-blade had wilted and that of the next leaf up.

(210.) Muskmelon. By the end of the ninth day the entire blade of the pricked leaf had wilted and changed color. The petiole of the pricked leaf was rigid. In 24 hours the wilt had increased somewhat but the petiole was still rigid. The following day the pricked leaf-blade and one coty-

ledon had begun to shrivel. The first leaf up showed a trace of flabbiness. The next afternoon the other cotyledon was drooping. The other leaves showed no sign of the wilt. Two days later the vine was bowed over at the root and all the foliage had wilted. When the stem was cut a stringy bacterial slime oozed out. The vessels were gorged with a schizomycete which had flooded out into the parenchyma. Some of the rods were distinctly larger (longer and broader) than the rest. The motility was not made out satisfactorily. Cultures were started from the interior of this vine and a section was saved in alcohol for study. (The cultures made were No. 9, a streak on agar, stock 82 c., and Nos. 10 to 14 on potato-cylinders.) The agar culture failed. With possibly one exception (tube 11), the inoculated tubes of potato developed pure cultures of *B. tracheiphilus*. At the end of 5 days the surface of the potato was covered by a very thin, exceedingly sticky layer, so exactly the color of the potato that it was to be distinguished from it only by its wet-shining appearance. In tube 11 most of the culture was of this character, but in the center there was a raised, slightly yellowish portion, believed to be a contamination.

(211.) Gherkin. By the ninth day the entire blade of the pricked leaf had wilted and changed color. The petiole was rigid. The blade of the first leaf up was wilted and the edges were rolled inward. The second leaf up was still green and turgid. Twenty-four hours later the cotyledons were hanging down and the second leaf above the pricked one was drooping. The following day there was little change, but 24 hours later the blades of all the leaves (it was a small plant) were badly wilted. The petioles were still rigid. Four days later the leaves had shriveled and also the base of the stem. The plant was brought in and dissected. Under the microscope, the vessels were found to be gorged with a sticky bacillus which strung out when touched. The tissues around the vessels were disorganized. The bacillus was motile and some of the rods were much larger than others—longer and especially broader.

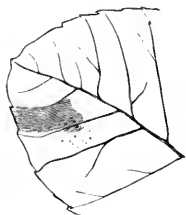


Fig. 71.\*

(212 a and b.) Gherkin. There were two vines in the same pot. One contracted the wilt and was removed on the seventh day when half of the pricked leaflet was wilted (2 p. m.). At that time the second vine was healthy but 2 days later (3 p. m.) it had two wilted leaves, the pricked one and the next one above it.

(213 and 214.) Gherkins. These damped off.

(215.) Winter Squash var. Pikes Peak. Two leaves were inoculated. The ninth day one of the pricked leaves had wilted outward from one of the groups of pricks and this area had changed to a light dull green. There were about twenty pricks in this group and the wilt did not include all of them (fig. 71). During the next three days there was little if any change. The sixteenth day about one-third of the pricked blade showed wilt and was drooping. There was also a small wilt-spot on the other leaf, including and surrounding the group of pricks. A week later the greater part of the blade of the lowest pricked leaf was flabby and yellow. The other inoculated leaf was dead in the pricked area, yellow around the pricks and slightly flabby on the whole of that side. November 11 (37 days after inoculation) the lowest leaf was dead and brown-shriveled down to the stem. The blade of the upper pricked leaf was shriveled and the petiole flabby at the apex and yellow its whole length. The blades of the next two leaves above were beginning to be a paler green. December 3 the vine was 13 inches long and had twelve leaves, those which had grown since the inoculation being dwarfish and not bright green. The next leaf up as well as the stem was very yellow. The plant was blossoming freely. December 10 the plant was still alive but stunted and yellowish (see photograph, fig. 53, made of this vine and a check). Thin sections were made of the stem and examined microscopically, the bacteria being detected in the bundles. Very few vessels were found to contain many bacteria. There was only one densely plugged vessel, and that was on the outer margin of the xylem near the cambium. The tissue was broken down on the outer margin of the xylem in several bundles. The cross-sections were not sticky. Stem, saved in alcohol. Sections cut from this stem show numerous bacteria in one or more vessels of five bundles but most of the vessels are free from them.

(216.) Winter Squash var. Pikes Peak. The ninth day after inoculation about 1 sq. cm. of the blade changed color and wilted, beginning in one of the three groups of pricks (fig. 72). The leaf was also slightly yellow around the other two groups of pricks. Twenty-four hours later the V-shaped small marginal piece of sound tissue was wilted, but otherwise there was little change. The sixteenth day about one-fourth of the pricked leaf was yellowish green and drooped slightly. Seven days later the portion above the line of October 21 (see drawing) was yellow and slightly flabby. The rest of the leaf was normal and there were no constitutional signs. November 11 (thirty-seven days after inocu-

\*Fig. 71.—Leaf of winter squash (plant No. 215) inoculated with *B. tracheiphilus*, Oct. 5, 1895. On Oct. 14 the shaded area was freshly wilted. Up to Oct. 17 there was little change, but on Oct. 21 about one-third of the leaf-blade was wilted and drooping. For further changes in this plant see fig. 53 A.

lation) the pricked leaf was holding up remarkably well. The petiole was yellowish but turgid, and not more than one-third of the blade was dead although it had lost nearly all of its green color and was yellow. The next leaf above and the next below were beginning to be yellow but showed no wilt. November 29 the vine was still alive but stunted and yellowish and losing leaves toward the base. It was blossoming freely. December 3 this vine resembled 215 except that the three basal leaves had shriveled and the others were not so yellow. The dwarfing of the foliage was distinct. March 4 (5 months after inoculation) the vine was still living but was stunted and branched and bore more yellow leaves than green ones. It and all the other squash vines had resisted remarkably.

(217.) Winter Squash var. Pikes Peak. There were no signs of the wilt until the twelfth day after inoculation. Then the tip of the pricked leaf was drooping (without change of color) and an area of about 2.5 sq. cm. had changed color and wilted, where pricked. Four days later there were very decided signs. The entire blade of the pricked leaf was drooping and over half of it had changed color, the change varying from a dull green to a yellowish. The blade of the next leaf below also drooped badly. The insertion of this leaf was half an inch below that of the pricked leaf. This was the only one of the squash vines which showed constitutional signs at this date (sixteenth day). The twenty-third day nearly all parts of the blade of the pricked leaf and of the one below it were shriveled. The blade of the next leaf up (insertion 1 inch above) was a fine green but was beginning to droop on one side—in the lower lobe. November 11 the blade of the pricked leaf was brown and dry but the petiole was green and turgid. No additional leaves were affected. November 29 the vine was still alive but stunted and yellowish and losing leaves at the base. It was blossoming freely. By December 3, the pricked leaf (with the exception of the base of the petiole) and the cotyledons had shriveled. The plant was 13 inches long and had 12 leaves. The stem was yellowish and the vine was dwarfed but was blossoming freely. December 31 (nearly 2 months after inoculation) the vine was still alive and, on the whole, looked better than 6 weeks earlier. It had made a new terminal growth, 12 to 15 cm. long, which was in every way more vigorous than the stunted, terminal growth which developed in the weeks immediately following the inoculation. The leaves were larger and of a good green and this part of the plant was blossoming. March 4 the vine was stunted and dead from the tip down a distance of about a foot. It had put out small stunted side shoots and there were some green leaves yet.

(218.) Winter Squash var. Pikes Peak. The ninth day after inoculation there was a wilted, dull-green area starting from two of the pricked spots. In one, the wilted part began at one side of the pricks, not including all of them, and covered an area of only 2 to 3 sq. mm. In the other the wilt now covered about 2 sq. cm. and involved all of the pricked spot although the latter was excentric. Twenty-four hours later the previously wilted tissue had lost water and begun to be traversed by many fine wrinkles. The diseased area had increased but little. The following day there was no noticeable change. The progress of the disease was very slow and on the sixteenth day not over one-eighth of the pricked blade had wilted and the blade as a whole had not collapsed. Even 7 days later (October 28) one set of pricks (No. 3) had failed to infect and that part of the leaf was still green. The tissue in the immediate vicinity of the other two sets of pricks was dry and brown. Most of the leaf was yellow or yellowish green and flabby but not shriveled. The petiole was normal. The first leaf up, the insertion of which was 2 inches above that of the pricked leaf, was still green but one lower lobe was drooping. November 11 half of the pricked blade was brown and shriveled, the rest was yellow and green mixed. All of it was soft, flabby. The petiole was still turgid and not very yellow. The blade of the next leaf up had a yellow spot on the upper part but was not flabby in any part. No other leaves were affected. November 29 the vine was stunted and yellowish and losing leaves toward the base but blossoming freely. December 3 the stem was 22 inches long and bore 16 leaves, the terminal 7 of which were badly dwarfed. The basal 2 leaves and cotyledons had shriveled to the stem. The foliage was lighter green than that of the uninoculated plants. A month later (December 31) the vine had made a new terminal growth 12 to 15 cm. long. This was of a much more vigorous character than

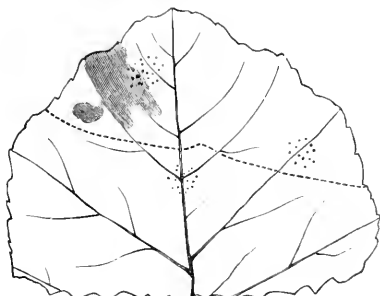


Fig. 72.\*

\*FIG. 72.—Leaf of squash No. 216, inoculated with *Bacillus tracheiphilus*, Oct. 5, 1895, 3 p. m., in three places. On Oct. 14, 3 p. m., the shaded area had changed to a dull green and wilted. Up to Oct. 17, 3 p. m., there was no distinct increase of wilt, but on Oct. 21, that part beyond the dotted line was yellowish green and drooped slightly. For further changes see text.

the stunted terminal growth which developed in the weeks immediately following the inoculations. The leaves were larger and of a good green and this portion of the plant was blossoming. March 4 the terminal 18 inches was dead. There were some good green leaves and the vine was still blossoming. It grew about 3 feet after inoculation.

(219.) Winter Squash var. Pikes Peak. This vine resisted admirably although the leaf received nearly 100 pricks. Twenty-three days after inoculation the leaf was yellow-green around the pricks and slightly flabby but there was no well-defined wilt. November 11 (37 days after inoculation) the blade of the pricked leaf and of the one next below were somewhat yellow-green but neither was flabby and there were no other signs. November 29 the vine was blossoming freely but was stunted and yellowish and losing leaves toward the base. December 3 the stem was 12 inches long and had 11 leaves. The basal leaf had shriveled nearly to the stem and the blade of the next up was yellow and shriveling. The stem and the foliage were light green. The foliage was dwarfed but the vine was blossoming profusely. This small plant had already borne 5 big blossoms (staminate). March 4 the vine was still in blossom. It had about 50 leaves, two-thirds of which were pale yellowish but not wilted. The rest were green.

(220.) Winter Squash var. Pike's Peak. The ninth day the pricked leaf-blade had changed color and wilted around two of the largest groups of pricks, while 24 hours later at least one-third of the pricked leaf drooped and nearly all of that part had become a lighter green. The wilted area on the opposite side of the leaf-blade (spot No. 2) had not sensibly increased. The following day wilt spot No. 2 had extended a little. There was no other change. Twenty-four hours later this side of the pricked blade drooped also and one of the cotyledons which had its origin at almost the same level as this leaf was beginning to droop. The petiole of the pricked leaf was still rigid. On October 21, the part which was wilted 4 days before was yellow and shriveling and hung down. The petiole and basal part of the blade were still green and turgid. A week later the pricked leaf-blade was brown and shriveled at the edges but green in the middle part and erect. There were no constitutional signs. November 11 the pricked leaf was yellowish-green in the middle of the blade. The petiole was still turgid. The blade of the next leaf up was yellow and shriveling at the tip. November 29 this vine resembled the preceding. December 3 it had 14 leaves and the stem was about 16 inches long. All except the basal leaves were dwarfed. Two of the latter had shriveled with the exception of the base of the petioles. It was blooming freely. The lower leaves were pale green. December 31 this vine resembled 219. March 4 the vine was still alive but stunted. It was branched and had some good leaves.

*Remarks.*—There was no sudden wilt of any of these squashes. A memorandum of October 14, states that "most of the squashes are taking the disease" but the final outcome shows that they proved very resistant in comparison with the cucumbers and muskmelons. Up to October 28, only two showed constitutional signs and in these the signs progressed slowly. Indeed on October 24, five of the six squashes appeared as if they were going to overcome the disease. This experiment indicates that the gherkin is also subject to the disease. In the inoculated squashes there was a tendency to profuse branching and blossoming. The same thing has been observed in the field (see p. 217). In general, infections are more certain when made from young pure cultures than when made with slime taken directly from the plant. On No. 220, the infections first appeared in the parts most severely injured, *i. e.*, where many needle pricks were made close together. Possibly to start the disease in squashes a larger initial injury is necessary than in case of cucumbers or muskmelons. Probably also, to be very sensitive, squashes must be growing rapidly and these were growing slowly. Query: Are the tissues more acid in slow-growing than in rapid-growing leaves? or more acid in squash than in cucumber? These hypotheses were formulated at the time as a partial explanation of the failures previously recorded. The squashes were planted in 6-inch pots in good soil. Query: Is the squash-disease due to an organism slightly different from the cucumber-organism?

#### INOCULATIONS OF NOVEMBER 16, 1895.

Three vines, a muskmelon, a cucumber and a squash, were inoculated at 4 p. m., with a white bacillus taken directly from the interior of a diseased cucumber-fruit grown in Barnabas Bryan's hothouse at Anacostia, D. C., and brought in to me for determination on November 14. The bundles of this fruit contained a very sticky stringy bacillus, great



quantities of which were pricked into the three plants. The cucumber-fruit was still green and firm but somewhat shrunken on the upper two-thirds and gummy drops had exuded and dried down. The flesh, except in the center, where the seeds were, had, in most places, a very decided water-soaked appearance but there was no soft-rot. The inoculations were made as follows: The freshly cut surfaces of the gummy fruit were pressed down gently on the leaf from opposite sides several times and then in each case about twenty needle-pricks were made through this moist surface.

(221.) Muskmelon (*Cucumis melo*). This was a small vine bearing six leaves. The pricked one was the third leaf up and its blade was about 2 inches broad. It was inoculated in two places, *i. e.*, there were two groups of pricks. The ninth day there were wilted areas centering in the pricked spots and covering in each case an area of about 1 sq. cm. The change of color (to a dull faded green) was decided. Four days later the petiole of the pricked leaf was still turgid. The blade of the first leaf up was slightly flabby. The next below was turgid but the blade of the second below had collapsed. This second below was the first leaf above the cotyledons and came out on the same side of the stem as the pricked leaf. Seventeen days after inoculation (December 3) the vine was badly wilted. The blades of all of the leaves had collapsed and the stem was beginning to shrivel near the base of the pricked leaf. It was now brought into the laboratory for minute examination. The stem was first cut near its juncture with the pricked leaf. The vessels here were full of bacilli some of which had flooded out into the parenchyma, but the cross-section was not sticky. The rods were not motile. An inch or two lower down the cut surface of the stem was sticky. An inch or two above the first cut, the vessels were crowded full. Some of the bacteria were motile. There also the cut surface was sticky. Cultures were made December 3 into tubes 5, 6, 7 and 8 of November 20 (which had failed).

(222.) Cucumber (*Cucumis sativus*). This vine was inoculated in the same way as the preceding. The pricked leaf was the third from the cotyledons and its blade was 4.25 inches broad. It received four sets of pricks, two on each side of the blade near its margin and about an inch apart. The ninth day on one side there was a wilt spot, about 2 sq. cm. in size, centering in one of the groups of pricks. On the other side there was a wilted area covering about  $5 \times 2$  cm., including both sets of pricks. The change of color was decided. It is probable that signs appeared on this and the preceding vine the previous afternoon. This would make the time of incubation 8 days. Two days later about half of the pricked blade had wilted. The thirteenth day nearly the whole of the pricked blade had shriveled and changed color. The petiole was turgid and all the rest of the plant was normal in appearance. The seventeenth day the vine was 15 inches long. The first four leaves above the pricked one were now flabby. The petiole of the pricked leaf and of the first two above it were still rigid. Those of the next two, which were younger and softer, were flabby. The uppermost wilted leaf was 8 inches above the node of the pricked leaf. The first leaf below (2 inches down) was turgid at 9 a. m. but at noon was slightly flabby on one side. On December 7, bacterial slime from this plant was used for further inoculations (see 226, etc.). Bacteria taken from the vessels were not clearly motile.

(223.) Winter Squash (*Cucurbita* sp.). One leaf was inoculated. Five sets of pricks were made, the method of inoculation being the same as that in the two preceding cases. The ninth day there were no signs. The eleventh day the tip of the pricked leaf had wilted over a space of about a square centimeter. The thirteenth day the tip of the pricked blade had recovered its turgor but was yellowish. The seventeenth day the vine was about two feet long and vigorous. It looked much as if the bacteria would not get out of the pricked leaf into the stem. The leaf still preserved its color and turgor except a few square centimeters at the tip of the blade which was yellowish and alternately turgid and flabby (see fig. 73). The dots indicate the pricked parts and the shaded apical portion the only part which was wilted at the time. December 13 (27 days after inoculation) the pricked areas were dead and the leaf was yellow around them. There was no wilt and most of the blade was green. December 31 three basal leaves including the pricked one, which had become gradually yellow, were shriveled but though I had watched this plant carefully for many weeks the disease gave no indication of spreading from the pricked leaf to other parts of the plant.

*Remarks.*—The bacillus used for these inoculations was taken, it will be remembered, from the interior of a green cucumber-fruit. In spite of its appearance I was in some doubt



Fig. 73.\*

\*Fig. 73.—Leaf of squash No. 223, inoculated with *Bacillus tracheiphilus* (cucumber strain) Nov. 16, 1895. Plant very resistant. The shaded area was wilted on Dec. 3. Less than natural size.

at first as to the nature of the organism in the bundles of this fruit because I had not hitherto known of the occurrence of the cucumber-wilt in hothouses, except as the result of my inoculations. It proved, however, to be infectious and yielded a long series of cultures and successful inoculations.

This experiment also shows that the squash is much more resistant than muskmelon or cucumber, at least to what I have come to call the cucumber strain.

#### INOCULATIONS OF NOVEMBER 29, 1895.

A muskmelon and a cucumber-vine were inoculated with *Bacillus tracheiphilus* from potato (?) tube 2, October 26 (from agar-stab of May 7). In each case many pricks were made on one leaf-blade.

(224.) Muskmelon (*Cucumis melo*). The eleventh day the pricked leaf-blade was partially wilted but the same was also true of two below and the cause was doubtful. Perhaps the wilt was due to the sulfuring done in the house some days before as other vines showed similar results. The seventeenth day it was still doubtful as to what was the cause of the wilt. December 31 the leaves were spotted and brown but it was doubtful if the bacteria were alive. January 7 the vine had shriveled down to the long hypocotyl which was still normal. It was now cut and examined in two places for bacteria but none were found.

(225.) Cucumber (*Cucumis sativus*). March 4 this vine showed no result from the inoculation.

*Remarks.*—The culture used was 34 days old, and probably dead.

#### INOCULATIONS OF DECEMBER 3, 1895.

Bacilli were squeezed out of the cut stem of a wilted muskmelon-vine (No. 221) and direct inoculations were made, at 3 p. m., into the following cucurbitaceous plants: common gourd (*Lagenaria vulgaris*); Balsam apple (*Momordica balsamina*); Gherkin (*Cucumis anguria*); vegetable sponge (*Luffa acutangula*); and wild gourd (*Cucurbita foetidissima*), a big rooted species native west of the Mississippi River. All of these were small plants, *i. e.*, only 3 to 8 inches high, but not stunted except the *Luffa* which was an older vine growing slowly and blossoming. The inoculations were made by means of groups of needle-pricks on the blade of one leaf. The bacteria used were sticky. Four days later these plants, all of which were growing nicely, were re-inoculated, each on another leaf-blade, using sticky bacterial slime out of the vessels of cucumber-vine No. 222. Great quantities of the bacteria were used. All of the leaf-blades of vine No. 222, from which these inoculations were made, had wilted and the interior of the stem was gorged with the bacteria. The vines were re-inoculated to make infection doubly certain. A limited area of the surface was first thoroughly wetted with the sticky slime and then many needle-pricks were made into this area. The inoculations were made in the hothouse.

(226.) Common Gourd. Twenty-eight days after inoculation (December 31), the plant had grown much and was blossoming freely, having shown no signs of the wilt. Three months after inoculation there was no general wilt.

(227.) Common Gourd. The eighth day at 1 p. m., the pricked leaf had changed color in the terminal part of the blade and two-thirds of this blade hung limp (it was normal at 10 a. m.). The period of incubation was a few hours less than 8 days. Five days later the blade of the pricked leaf had wholly collapsed and part of it was yellowish. The next leaf below (the one which was re-inoculated December 7), was still normal. About 3 weeks after inoculation the wilted leaf had shriveled to the stem but none of the others showed any indications of wilt although the first internode up was only half an inch long and that next below was only 0.75 inch. January 10 (38 days after inoculation) the next four leaves above the pricked one and also one below had collapsed. Previous to this there had been no indication of the disease (except wilt of these four leaves for a few hours only on December 16, ascribed to lack of water), and I thought the plant had overcome the bacteria. Careful examination of the stem in two places, *i. e.*, just above and below the pricked leaf, showed no bacteria and the wilt was not accounted for.

(228.) Common Gourd. The plant grew and blossomed freely. The inoculation produced no disease.

(229.) Common Gourd. This plant was like the preceding. With the exception of vine 227, all the inoculations into *Lagenaria* failed to induce anything more than trivial local injuries. They were inoculated for the third time on January 7 and numbered 260-262 (q.v.).

(230.) Balsam apple. The tenth day there were no signs of the wilt, but 3 days later both pricked leaves showed very small wilted areas. December 31 the pricked portions were dead and had narrow yellow borders, but the rest of the leaf was normal, green, and turgid and there were no indications of any secondary wilt. The plant was growing and had five times as much leaf-surface as when pricked. The inoculation failed to induce anything more than local injuries and the vine was re-inoculated January 7 and numbered 263 (q.v.).

(231.) Balsam apple. Like the preceding. Re-inoculated and renumbered (264.) on January 7.

(232.) Balsam apple. The seventh day there seemed to be a little wilt around some of the pricks first made. The following day there was a plain case of wilt with change of color to a dull green on the leaf-blade pricked December 3. About one-fourth of the outer part of the leaf had drooped. The thirteenth day the wilt was increasing slowly on the first pricked leaf and a wilt-spot of a few square millimeters had developed on the other pricked leaf (pricks of December 7). The twenty-eighth day the inoculated leaf which had shown distinct wilt where pricked had dried out. Half of this blade (the pricked part) was dead. The rest was a good green. The plant had grown well and had five times as much leaf-surface as when pricked. It was re-inoculated January 7, and numbered 265 (q.v.).

(233.) *Cucumis anguria*. The sixth day there were no signs but the following morning both the pricked leaves and the one next above were badly wilted. The eighth day this vine had developed a bad case of the bacterial wilt. The thirteenth day the plant, which was a small one, had collapsed.

(234.) *Cucumis anguria*. The sixth day there was distinct wilt on the blade of the leaf first pricked, also on the first leaf up and the first down (pricked December 7). It was 5½ days since the first inoculation. Two days later this plant was badly wilted and the thirteenth day it had collapsed.

(235.) *Luffa acutangula*. The eighth day the plant was normal except for a little yellowing around the groups of pricks made on December 3. The tenth day there were no signs of wilt. Three days later both leaves were yellowish around the pricks but there was still no wilt. On December 31 (28 days after inoculation) the pricked areas were dead and had yellow borders but there had been no wilt even of the pricked leaves. The vine had doubled its size since it was pricked. On January 7 it was re-inoculated and numbered 266 (q.v.).

(236.) *Cucurbita foetidissima*. The sixth day the leaf inoculated on December 3 had wilted and changed to a dull green where pricked so that the terminal third of the leaf-blade hung down flabby. Twenty-five hours later the next leaf down (pricked December 7), was beginning to show a trace of wilt. The following day the blade of the first leaf up and of the first below the pricked leaves drooped. Thirteen days after inoculation (December 16) all the foliage had shriveled. The plant was a small one. Two days later it was brought in and examined microscopically. Cross-sections of the stem were not noticeably sticky but the interior of the vessels and surrounding parenchyma were gorged with bacilli. At least one-tenth of the rods were noticeably larger than the rest. The largest were estimated to be ten times larger than the smallest—longer and broader. None were clearly motile. Material saved in alcohol.

(237.) *Cucurbita foetidissima*. The sixth day there were no signs of the disease but 2 days later the blade of the leaf inoculated on December 3 showed a trace of wilt at the apex where it was pricked and also a slight change of color. The period of incubation was nearly 8 days. The following afternoon the blade of the leaf first pricked was badly wilted. The next morning the blade of the second pricked leaf was half wilted. Three days later (13 days after inoculation) all the foliage of this vine which was a small one, was shriveled. The fifteenth day it was brought in and examined microscopically. The vessels and surrounding parenchyma were crowded with bacilli which were like those found in the preceding. The cross-sections were not noticeably sticky. Material saved in alcohol.

(238.) *Cucurbita foetidissima*. The sixth day the blade of the leaf which was first pricked had changed to a light green and wilted in tiny spots around three of the four groups of pricks. Twenty-five hours later the terminal half of the leaf first pricked had changed color and was drooping. The following day the first leaf above and the first below were wilted, the petioles of the lower two leaves being involved in the flaccidity. The thirteenth day the foliage had shriveled. The vine was a small one.

*Remarks.*—*Cucumis anguria* and *Cucurbita foetidissima* contracted the disease promptly and proved as sensitive as cucumber or muskmelon. *Luffa*, *Momordica*, and *Lagenaria* were resistant.

## INOCULATIONS OF DECEMBER 7, 1895.

Inoculations were made at 2 p. m., into gherkin (*Cucumis anguria*), cow-pea (*Vigna*), muskmelon (*Cucumis melo*), and cucumber (*Cucumis sativus*), using sticky bacterial slime out of the vessels of cucumber-vine No. 222. Great quantities of the bacteria were used. A limited area of the surface was first thoroughly wetted with the sticky slime and then many needle-pricks were made into this portion.

(239.) Gherkin. Four days after inoculation (between 10 a. m. and 1 p. m.) the tip of the pricked leaf changed color and commenced to wilt. The ninth day the plant, which was a small one, had collapsed.

(240.) Cow-pea. The twenty-fourth day there was no wilt or death of the tissue. Up to March 4, there had been no result from the inoculation.

(241.) Muskmelon. The third day there was a small wilted spot including each of the five sets of pricks. An enormous number of bacteria were pricked in and this was supposed to account for the unusually short time between inoculation and the first signs, *i. e.*, only 68 hours. There was little increase of the wilt, however, during the next 24 hours. On the sixth day signs were uncertain. The twenty-fourth day the leaves were spotted and the vines were not thrifty but it was doubtful if the bacteria were still alive. Up to March 4 there had been no other result from the inoculation.

(242.) Cucumber. This was a large plant. The fourth day the vine showed no signs of the wilt. Two days later the case was doubtful but the ninth day the blade of the pricked leaf was dry-shriveling. The twelfth day the leaves above and below were turgid. Twenty-four days after inoculation (December 31), the stem was collapsing, all the leaves having shriveled some days ago. The stem was now cut open and the interior found to be full of a white sticky mass of bacteria. Inoculations into 244 and other vines were made direct from the interior of this plant. A loop of slime taken from the interior of 242, with bacteriological precautions, and spread on slant agar, yielded a pure culture which was used to inoculate No. 251, etc.

(243.) Cucumber. This was a big plant. The fourth day (1 p. m.) the inoculated leaf had changed to a pale green and had begun to wilt around one of the five groups of pricks. The period of incubation was nearly four days. Two days later one-fifth of the pricked blade hung flabby and had become a dull green. The ninth day the blade of the pricked leaf was shriveling. The twelfth day three leaves above the pricked one were wilted. They had been turgid the day before. The first leaf below was normal. The twenty-fourth day the stem was collapsing. All the leaves had shriveled some days ago.

*Remarks.*—The cow-pea did not contract the disease. The three closely related species of curcubits contracted the disease.

## INOCULATIONS OF DECEMBER 31, 1895.

Three cucumbers (*Cucumis sativus*) and four squashes (*Cucurbita maxima*) were inoculated in the hothouse, at 9 a. m., with a white sticky mass of bacilli from the interior of cucumber-vine 242 (cucumber-strain). The bacterial slime was pressed out on the leaves and then pricked in with a sterile steel needle. Many pricks were made. In some cases the bacteria were put on the dorsal side, in others on the ventral side of the leaf. Each of the cucumbers was inoculated on one leaf, each of the squashes was inoculated on one green cotyledon.

(244.) Cucumber. The eighth day (10 a. m.) there was wilt on one margin of the inoculated leaf extending outward from three groups of pricks— $5 \times 1$  cm. The blade of this leaf was 4 inches broad. There had been no wilt the preceding afternoon. The tenth day the whole of the pricked blade was wilted. Other leaves were normal. The sixteenth day the petiole of the pricked leaf was still turgid to the tip but the blade had dry-shriveled. All the leaves above the pricked one, four in number, were wilted. The blade of the first leaf down was also wilted but the others were turgid. The twenty-fifth day the apical part of the stem had shriveled and the disease was slowly passing downward. The vine finally shriveled to the ground.

(245.) Cucumber. There were no signs until the ninth day. Then the apex of the pricked leaf was wilted. The following day the apical half of the leaf had changed to a dull, faintly yellowish green and had wilted, the wilt clearly commencing in the pricked areas (see figure 74). The rest of the leaves were normal. On the sixteenth day the blade of the pricked leaf was shriveled; the petiole was turgid except toward the top where it was a trifle flabby. The upper five leaves were wilted and also the

two next below the pricked leaf. The rest were normal. The twenty-fifth day the apical 10 inches including three internodes below the pricked leaf were dry-shriveled. The wilt was slowly extending downward. Another big leaf-blade, the fourth down, had drooped the preceding day. Farther down were five good leaves separated by long internodes. The stem was horizontal. The whole vine finally shriveled to the ground.

(246.) Cucumber. The eighth day (10 a.m.) there was wilt of the apex and margins of the pricked leaf, extending outward mostly from the groups of pricks, involving 10 to 12 sq. cm. The leaf was 4 inches broad. There had been no wilt up to the preceding afternoon, therefore the period of incubation was nearly eight days. The tenth day the terminal three-fourths of the pricked blade had wilted and was a dull yellowish green (it was more green than yellow but looked faded). The petiole and remaining leaves were normal. By January 16 the blade of the pricked leaf had shriveled. The upper half of the petiole was flabby and the extreme upper end was shriveling. The terminal six leaves (6 inches of stem) had wilted and also the blade of the first leaf down, the separating internode being 4 cm. long. The rest were normal. The twenty-first day the upper half of the vine had wilted. It was now brought into the laboratory and by direct transfer four slant agar-cultures were made from it (tubes 1 to 4, January 21, 1896) as follows:

No. 1 from base of wilted part of stem (interior);

No. 2 from interior of a young shriveling fruit near the top of the vine;

No. 3 from middle of wilted part of the stem which had begun to shrivel;

No. 4 from interior of stem not far from the lowest external sign of wilt (in leaf) and where the stem was sound externally but sticky within. Nos. 1 and 4 yielded pure cultures of *Bacillus tracheiphilus*.

(247.) Squash. Groups of pricks were made on a big cotyledon. Up to March 4 there had been no general wilt. The pricked cotyledon was dead and the pricked part was thicker than the rest as if from development of cork-tissue.

(248.) Squash. One of the cotyledons was pricked. There was no result from the inoculation. Even the cotyledons did not wilt. There seemed to be cork in and around the pricked area.

(249.) Squash. The pricks were made in one of the cotyledons. Up to March 4 (63 days) there were no general signs resulting from the inoculation, although 22 days after inoculation there was a decided wilt at the tip of the cotyledon which continued for several days extending very slowly from the group of pricks outward.

(250.) Squash. This vine was also pricked on one of the cotyledons but with no result. On March 4 the pricked part looked as if cork-tissue had been formed there.

*Remarks.*—In the cucumbers the bacterial wilt progressed upward faster than downward, *i. e.*, two or three times as fast. On the sixteenth day in the squash all the pricked cotyledons were large, thick and green. One of the pricked cotyledons wilted at the tip after 22 days.

One of the cotyledons, collected March 4 (probably from 247 or 249) was afterwards infiltrated with paraffin and sectioned. Bacteria were present in some of the bundles and some of the latter were a little disorganized but not much. The impression one gets from these sections is that the bacteria have multiplied in the vessels *very slowly*. A definite corklayer was not made out.

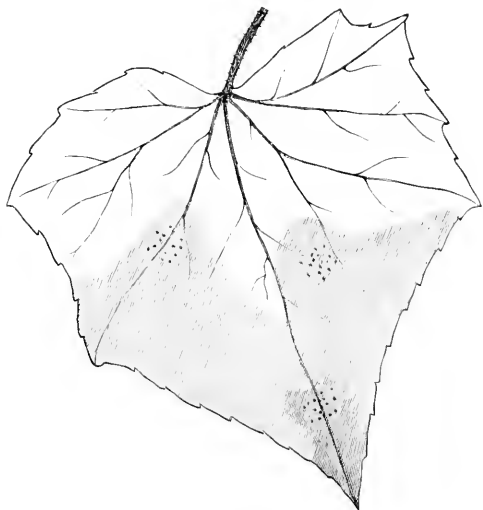


Fig. 74.\*

\*Fig. 74.—Leaf of *Cucumis sativus* (plant No. 245) inoculated with *B. tracheiphilus* and shaded to show progress of wilt. The needle-pricks were made Dec. 31, 1895. First sign of disease appeared at apex of leaf on ninth day. About 24 hours later wilt had extended as indicated by lighter shading. Drawn by the writer.

## INOCULATIONS OF JANUARY 7, 1896.

A set of inoculations was made in the hothouse at noon on cucumber (*Cucumis sativus*), pumpkin (*Cucurbita pepo*), squash (*Cucurbita maxima*), cow-pea (*Vigna*), tobacco (*Nicotiana tabacum*), common gourd (*Lagenaria vulgaris*), balsam apple (*Momordica balsamina*), and the vegetable sponge (*Luffa acutangula*). The bacteria used were from a thin, nearly colorless, wet-shining, very sticky growth (cucumber-strain) covering nearly the whole surface of a slant agar (tube No. 4, December 31, which was inoculated from the interior of vine 242 and yielded a pure culture). The inoculations were made with a sharp-pointed steel-needle and the culture was so sticky that an enormous number of bacteria must have been inserted into the leaves.

(251.) Cucumber. This was a large vine. Many pricks were made on one leaf. The wilt appeared on the seventh day in the pricked area to which it was then confined. Two days later the wilt had moved slowly from the pricked area, and now involved about one-seventh of the blade—the terminal portion from the pricked area to each side and outward. The wilt had extended more rapidly up than down. The rest of the vine was normal. The eighteenth day the terminal part of the stem had dry-shriveled and the wilt was slowly passing downward. The whole vine finally shriveled to the ground.

(252.) Cucumber. No pricks were made but a big loop of the sticky bacterial slime was put into a fresh pistillate flower on and below the stigmas. The flower was near the apex of the stem. The eighteenth day there was some possibility that the bacteria had passed into the fruit. It had changed color throughout, *i. e.*, it had become a dirty green. The fruit was 0.5 to 0.75 inch long at this time. There had been no wilt of the leaves as yet. Up to March 4, however, there were no further signs.

(253.) Pumpkin-seedling. Many pricks were made on a cotyledon. The inoculation failed. Not even the whole of the cotyledon wilted.

(254.) Pumpkin-seedling. Many pricks were made on the blade of the tender first leaf. The thirteenth day the pricked part of the leaf was wrinkled and yellowish but not wilted. Five days later one side of the pricked leaf had wilted. By March 4 (56 days) the pricked leaf had wilted to the stem but there were no other signs of disease and the plant was very thrifty.

(255.) Pumpkin-seedling. Many pricks were made on the small, delicate first leaf. The eighth day the pricked apex of the blade was wilted and the following day the wilt involved about one-fourth of the leaf. Four days later the pricked part of the leaf had dry-shriveled and the wilt was extending, although the middle basal part of the leaf was still turgid as was also the petiole. Twenty-four hours later the upper part of the petiole had drooped over. The eighteenth day the pricked leaf had wilted and shriveled nearly to the stem and the cotyledons were beginning to droop. The whole vine ultimately shriveled to the ground.

(256.) Pumpkin-seedling. Many pricks were made on the tender, partially developed first leaf. The thirteenth day about two-thirds of the blade of the pricked leaf had wilted, *i. e.*, the pricked apical area and the margins nearly to the petiole. Five days later the pricked blade and over half of the petiole had shriveled. The whole vine shriveled to the ground after a time.

(257.) Winter-squash-seedling. Many pricks were made on one of the big green cotyledons. The thirteenth day there was a distinct wilt and loss of color extending from one group of pricks to the edge of the leaf—about 1 sq. cm. Five days later there was a bad droop of the pricked cotyledon. Up to March 4, however, there had been no general wilt and the plant was still thrifty. Not even all of the pricked cotyledon had wilted.

(258.) Cowpea. This plant was 6 inches high and was just developing the third true leaf. Many pricks were made on the apex of one of the first leaves.

There was no result from the inoculation.

(259.) Tobacco var. Little Oronoco. Many pricks were made on the apex of the blade of a bright green leaf, 3×1.5 inches.

There was no result from the inoculation.

(260.) Common gourd (No. 226 of December 3, now inoculated for the third time). Many pricks were made on the apex of the leaf-blade midway up. The seventh day there was a slight flabbiness at the tip of the pricked leaf. Two days later there had been no decided change. Up to March 4 there was no general wilt.

(261.) Common gourd (No. 228 of December 3, now inoculated for the third time). Many pricks were made on the apex of a leaf-blade midway up on the stem. The seventh day there was a slight flabbiness at the tip of the pricked leaf. The ninth day there was no decided change and the case was considered a doubtful one. On March 4 the plant was still living and there had been no general wilt.

(262.) Common gourd (No. 229 of December 3, now inoculated for the third time). Many pricks were made on the blade of a leaf half-way up the stem. The ninth day there had been no wilt. Up to March 4 there was no general wilt.

(263.) Balsam apple (No. 230 of December 3, now inoculated for the third time). Many pricks were made on a leaf half-way up. This was a small plant on December 3, when it was first inoculated but now it was about 16 inches long with 17 leaves. The others had grown nearly as much. The seventh day there was wilt around the pricks. This had appeared probably the day before. Two days later the wilted area extended out a short distance from the pricked area. Up to March 4 (56 days) there had been no general wilt and the plant was still growing slowly.

(264.) Balsam apple (No. 231 of December 3, now inoculated for the third time). Many pricks were made on a bright green leaf half-way up the stem. The seventh day there was wilt around the pricks which, however, increased little if any during the next 2 days. Up to March 4 there had been no general infection. Not even all of the pricked leaves wilted: They only became somewhat yellowish.

(265.) Balsam apple (No. 232 of December 3, now inoculated for the third time). Many pricks were made on the blade of a bright green leaf half-way up the stem. The seventh day there was a wilted area around the pricks. Two days later there was little, if any, increase in this area. On March 4 the plant was still healthy.

(266.) *Luffa acutangula* (No. 235 of December 3, now inoculated for the third time). Many pricks were made on a leaf-blade near the apex. On January 16 the plant was healthy. Up to March 4 there was no general wilt.

*Remarks.*—Cow-pea, tobacco, and *Luffa* refused to take the disease. Pumpkin, squash, balsam-apple, and gourd showed local signs but even the pricked leaves did not succumb as a whole and no secondary or general signs appeared, *i. e.*, there was no wilt outside of the pricked leaf except perhaps in Nos. 255 and 256. Cucumber (No. 251) contracted the disease.

#### INOCULATIONS OF JANUARY 21, 1896.

Inoculations were made in the hothouse at 10 a. m., on *Cucumis sativus*, *Cucurbita californica* and *Cucumis anguria*. The bacillus was taken from tube 6 December 31, the bulk of which had been used up the preceding day in inoculating fermentation-tubes and making new agar-cultures. The bacteria were introduced by needle-pricks. The plants were under observation until March 4.

(267 and 268.) *Cucumis sativus*. These were old vines. Many pricks were made on a leaf-blade. No signs appeared.

(269.) *Cucurbita californica*. Many pricks were made in the fourth leaf-blade above the cotyledons. The vine was a young one.

There was no result from the inoculation.

(270 and 271.) Young *Cucumis anguria*. Many pricks were made on a leaf-blade.

There was no result from the inoculation on either plant.

*Remarks.*—These five inoculations were made into plants of three susceptible species. All failed. The reason for this failure is to be sought in the nature of the culture used. What was then puzzling is now perfectly plain. The culture was 21 days old, and was on stock 93 b, an agar to which sugar had been added. On the same date as these plant inoculations, four fermentation-tubes were inoculated copiously from the same culture and all failed (7 days test) showing that the culture was dead. The body of the fluid in these tubes consisted of slightly alkaline (litmus) peptonized beef-broth free from muscle-sugar. One of these fermentation tubes contained saccharose, another maltose, another lactose and the fourth dextrine, each of these very suitable foods being added in the proportion of 0.2 per cent. The early death of the culture was undoubtedly due to the injurious action of its own by-products, *i. e.*, of acids derived from the decomposition of the sugar, this organism being extremely sensitive to acids.

INOCULATIONS OF FEBRUARY 26, 1896.

A set of inoculations was made in the hothouse at 2 p. m., from a white, wet-shining, sticky culture of *Bacillus tracheiphilus* on steamed carrot (tube 2 February 18, from 1, February 13 which was a potato culture made from a fermentation-tube (No. 1, January 20) containing cane-sugar. The plants inoculated were: *Benincasa cerifera*, *Cucurbita foetidissima*, *Cucurbita californica*, cucumber, muskmelon, watermelon and *Datura stramonium*. Numerous bacteria were put in with each inoculation, which was made with a sharp-pointed steel needle. All of the plants were examined on February 28 and February 29, and were free from disease.

(272.) *Benincasa cerifera*. This was a small plant having three leaves besides the green cotyledons. Many pricks were made on the middle leaf. The plant appeared healthy on March 5. The first signs were noted on March 6 at 2 p. m. (end of the eighth day) at which time there was a slight wilt extending outward from the pricks to the margin. The following day fully half of the pricked leaf had wilted. Two days later the whole of the blade of the pricked leaf had wilted, as also that of the first leaf above and below. The sixteenth day the leaves were badly shriveled, but the stem was normal. Material was saved in alcohol. On microscopic examination enormous numbers of bacteria were found in the vessels (slide No. 202).

(273.) *Cucurbita foetidissima*. This vine was grown from seed planted October 11, and at the time of inoculation had eight good leaves. Many pricks were made on the under side of an upper leaf. The fifth day (9 a. m.) the leaf looked suspicious and at 1<sup>h</sup>20<sup>m</sup> p. m. there was distinct wilt at the tip and a dull green color where pricked. The following day there was little change. The next day the pricked area had changed to a whitish green and the terminal one-fifth of the blade hung flaccid. The following day about one-third of the leaf was wilted and 28 hours later (close of eighth day) there was a bad collapse of two-thirds of the pricked blade. The beginning of the eleventh day the whole of the blade of the pricked leaf had wilted but the petiole was still rigid. The blades of the first and second leaf up were now inclined to droop. Two days later the blade of the first leaf down was wilted. All the petioles were still rigid. The sixteenth day all the leaf blades had shriveled. The stem and petioles (lower two-thirds) were normal. The plant was put into alcohol. On microscopic examination the bundles of the petiole of the inoculated leaf (fig. 77) were found filled with bacteria and badly disorganized (slide No. 254). The bacteria also extended into the bundles of the fleshy root but here the disorganization was less.

(274.) Cucumber (*Cucumis sativus*). This was a thrifty young plant. Many pricks were made on the tip of an upper leaf. The fifth day (9 a. m.) there was wilt and change of color in an area of 10×3 mm., along one side of the pricked area which was about 10×10 mm. in diameter. At 1<sup>h</sup> 20<sup>m</sup> p. m. of the same day the wilted area was twelve times as large. The following morning there was little change, but 24 hours later the terminal eighth of the leaf was drooping. The next day the change of color in the wilted area was more decided but the latter had not increased much. At the end of the ninth day there was very striking wilt confined to the terminal half of the pricked leaf, a wedge-shaped area 7.5 cm. long by 2.5 cm. wide (in the widest portion). It was dull green and the tip was drooping. Twenty-five hours later the whole of the blade of the pricked leaf had wilted. The rest of the vine was normal. On March 9, the blades of the first two leaves above and the first two below had wilted but the petiole of the pricked leaf was still rigid. Five days later (seventeenth day) the vine was badly wilted and was pulled up for microscopic examination. The stem was green and turgid but its vessels contained a sticky bacterial slime which strung out in long, delicate threads. The middle part of the stem was saved in alcohol.

(275.) Cucumber. This was an old plant. Many pricks were made on the blade of an old, upper, whitish leaf. On March 3, at 10 a. m., there were no signs but 24 hours later half of the leaf was flabby and drooping, on the pricked side. It was now nearly 7 days since the leaf was pricked. The following day the whole of the pricked blade was dry shriveled and the tip of the petiole was slightly flabby. On March 6 (end of ninth day) the petiole had shriveled. The rest of the vine was normal. The following day there were constitutional signs. By 10 a. m. of March 9 everything had collapsed except the green stem and a few small basal leaves.

(276.) *Cucumis melo* var. *dudaim*. This was a small plant. Many pricks were made on one leaf-blade. The fifth day there was slight wilt in the pricked portion and running out to one margin of the blade. The following day there was little change, but 24 hours later the whole pricked leaf was drooping. This, however, was favored by a dry soil because, on watering, the leaf recovered its turgor in the afternoon with the exception of the tissue immediately around the pricks. The next morning the pricked leaf was turgid with the exception of a wedge-shaped piece extending from the



pricks to the margin. The ninth day (2 p.m.) the wilt involved most of the pricked side of the leaf (fig. 75) and 25 hours later all of the pricked leaf had wilted but the petiole, while the other leaves were normal. The eleventh day all the leaves had collapsed. The sixteenth day the vine had shriveled to the ground.

(277.) *Cucumis melo* var. *dudaim*. This was a small plant. Many pricks were made on the blade of one leaf. The sixth day (10 a. m.) there was a slight wilt and change of color in the center of the pricked area. Twenty-four hours later two-thirds of the pricked leaf was drooping. The eighth day the pricked leaf was turgid with the exception of a wedge-shaped area extending from the pricks to the tip and involving about one-eighth of the blade (fig. 75). The next afternoon there was only a little increase of the wilt, but 25 hours later all of the blade of the pricked leaf was wilted. The petiole was still turgid. The blades of the first three leaves up now showed a slight droop. The twelfth day all the leaves had collapsed and the sixteenth day the vine had shriveled to the earth.

(278.) *Benincasa cerifera*. Many pricks were made on one of the leaf-blades of a small plant. On March 3 there were no signs, but 24 hours later there seemed to be a slight droop of the pricked portion. On March 5 there was no clear evidence of the wilt, but the following afternoon (end of the eighth day) there was change of color and distinct wilt. These signs were confined to an area of about 1 sq. cm. from the pricks to the tip, and the most of the pricked leaf was still normal. Twenty-five hours later the wilt was spreading slowly in the blade of the pricked leaf. The twelfth day six leaves besides the pricked one (part above it and part below) were wilted, some badly. Four days later the vine had shriveled to the ground.

(279.) Watermelon (*Citrullus vulgaris*). This was a small plant. Many pricks were made on one leaf-blade. On March 3, at 10 a. m., there were no signs of the wilt, but 24 hours later the pricked leaf had changed color and wilted from the pricked area outward to the tip, about one-third of the leaf being affected. The following day the leaf had recovered its turgor except a very small wedge at the tip beyond the pricks. The bulk of the pricked area seemed normal. The next afternoon there was no decided increase.

During the next 25 hours there was a slow spread of the wilt and change of color (whiter) in the blade of the pricked leaf. Two days later about one-fourth of the pricked leaf had wilted and dried out. The rest was normal. Up to the seventeenth day there was no change. Four days later the whole of the blade of the pricked leaf, which was a small one, had wilted. The petiole and remainder of the plant were normal.

(280.) *Cucurbita californica*. Many pricks were made on one leaf-blade which was 2 inches across. The leaf was almost exactly the shape of a leaf of English Ivy. On March 3, at 10 a. m. there was a slight wilt in the pricked area. Twenty-four hours later about one-third of the pricked leaf had wilted and the following morning fully one-half of the inoculated leaf had succumbed to the wilt (see fig. 76). The next afternoon (March 6) the whole of the pricked leaf had collapsed, also the first leaf below, the neighboring cotyledon, and the first three leaves above. The stem was turgid as were also the fourth and fifth leaves up and the other cotyledon. Twenty-four hours later the signs were much aggravated. The twelfth day the leaves had collapsed

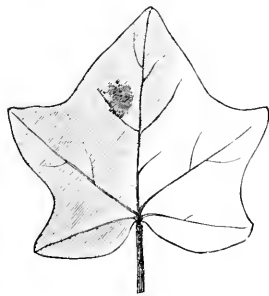


Fig. 76.†

including the petioles and the terminal part of the stem. The plant was now removed and put into alcohol. On microscopic examination great numbers of bacteria were found in the vascular bundles of the stem.

(281.) *Datura stramonium*. This was a young thrifty plant. Many pricks were made on a leaf-blade. A great quantity of bacteria were put into the leaf, but up to the twenty-first day the plant was growing finely and there were no signs of the wilt.

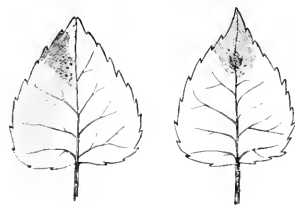


Fig. 75.\*

\*Fig. 75.—Left: Leaf of plant No. 276 (*Cucumis melo* var. *dudaim*), ninth day after inoculation with *Bacillus tracheiphilus*, shaded part wilted. Right: Leaf of inoculated plant No. 277 (*Cucumis melo* var. *dudaim*) on eighth day after *Bacillus tracheiphilus* was introduced by needle-pricks. The first wilt was a little earlier (central dark shading).

†Fig. 76.—Leaf of *Cucurbita californica* (plant No. 280) inoculated with *B. tracheiphilus*. The needle-pricks were made Feb. 26, 1896, and the wilt appeared in the deeply shaded part March 3. During the next two days it involved over half the leaf-blade as shown by the lighter shading. The whole plant collapsed on the twelfth day.

*Remarks.*—*Datura stramonium* resisted. Local signs were obtained on the watermelon but there was no general infection of the plant. Local and then constitutional signs appeared on the cucumbers, on *Cucurbita foetidissima*, *C. californica*, *Benincasa cerifera* and on the little melon, *Cucumis melo* var. *dudaim*. The old and young cucumber proved equally subject to this disease.

The bitter plant which I have called *Cucurbita californica* was grown from seeds sent to Mr. Gilbert Hicks by Prof. J. W. Toumey of Arizona. It came to me unnamed and I had much difficulty in classifying it. The plant was finally determined for me by Dr. J. N. Rose of the U. S. National Herbarium.

#### INOCULATIONS OF APRIL 4, 1896.

Four vines of *Melothria scabra*, three of cucumber, one of *Echinocystis lobata*, one of watermelon and two of *Cucumis crinaccus* were inoculated with a pure culture of *Bacillus tracheiphilus* taken from tube 1, March 30. A big loopful of the bacterial slime was put on one leaf of each plant and pricked in with numerous fine punctures, using a small sharp steel needle. In some cases an additional loop of the slime was afterwards put on over the pricks which were protected from direct sunshine. The rods in this tube were mostly in a state of active motility as determined by examination in a hanging drop. Inoculations in each case were made on the blade of the leaf and were very thorough.

(282.) *Melothria scabra* (from Mexico). Up to April 13 there were no signs. The eleventh day there was a yellowing of the tissue about the pricks, but no wilt of the blade. Two days later there was little change (the weather for the past six days had been very hot). The twenty-fourth day the pricked leaf had shriveled but the rest of the plant was normal. On June 16 the plant was still alive the inoculation having failed to kill it. On June 25 (82 days) there was still no result other than the local injury.

(283.) *Melothria scabra*. There were no signs until the eleventh day. At that time a very little of the tissue in the pricked area was dead (1 to 2 sq. mm.) but there was no general wilt of the leaf. Two days later the pricked leaf was yellowish but not wilted. The twenty-fourth day the pricked leaf had shriveled but none of the others showed any trace of the wilt. On June 16 the plant was still living. The inoculations failed to induce constitutional signs.

(284.) *Melothria scabra*. On April 13 the plant was normal. The eleventh day the leaf-blade was yellowish green and puffed out where pricked. Two days later the pricked leaf was yellow around the pricks but not wilted. The twenty-fourth day the pricked leaf which had shown itself very resistant at first, had shriveled. The other leaves were normal. June 16 the plant was still living and did not show any constitutional signs.

(285.) *Melothria scabra*. This plant behaved like the preceding. The inoculation did not harm the plant beyond the pricked leaf.

(286.) Common Cucumber. No record earlier than April 13. On that day there was wilt on one side of the leaf around the pricks. Two days later the whole blade of the pricked leaf was drooping. The thirteenth day the whole of the pricked leaf-blade had wilted, also two leaves above and one leaf below. The petioles were turgid. The twenty-fourth day the plant was dead.

(287.) Common Cucumber. No record earlier than April 13. On that day there were no signs of the disease, but 2 days later there were a few square centimeters of wilted tissue in and around the pricks. The thirteenth day the whole of the pricked blade was wilted. The leaves above and below were normal. The twenty-fourth day the stem was still green, but the leaves had wilted.

(288.) Common Cucumber. The ninth day the pricked leaf was normal, but 2 days later there was wilt of a few square centimeters in and around the pricked area. The thirteenth day the whole of the pricked leaf had wilted. The leaves above and below were normal. The twenty-fourth day the plant was dead.

(289.) *Echinocystis lobata*. No record earlier than April 13. The afternoon of that day there was wilt of the tip of the leaf, *i. e.*, of the tissue in and around the pricked area. There was only a slight change the following noon. The eleventh day most of the pricked leaf was turgid. The wilted portion had dried out and apparently the disease had come to a stop. Two days later there was no increase of the wilt and the greater part of the pricked leaf was normal. The twenty-fourth day the plant was growing rapidly and had recovered. On June 16 the plant was still living. It had made a long growth and blossomed. Only a small part of the pricked leaf succumbed.

(290.) Common Watermelon. The ninth day there was wilt of the tip of the leaf beyond the pricks and reaching down to them. There was only a slight change the following noon, and the eleventh day the wilt seemed to have stopped. The tip of the leaf beyond the pricks was dead (a few sq. mm.) but between them the tissue was still living, although a yellowish green color. Two days later all but about one-twenty-fifth of the pricked leaf-blade was normal. There had been little change since the eleventh day. The twenty-fourth day the wilted portion of the leaf was brown and dry but there had been no increase of the disease.

(291.) *Cucumis erinaceus*. The ninth day there was wilt in the pricked area but the remainder of the leaf-blade was turgid. The following noon there was no change. The eleventh day there was a small dead patch in the pricked portion, but no general wilt of the leaf. The terminal portion had recovered its turgor. On April 17 the terminal one-sixth of the leaf in and beyond the pricked portion was wilted, but the rest of the plant was normal. The twenty-fourth day the plant had recovered and was growing. June 25 the plant had fully recovered and was making a fine growth.

(292.) *Cucumis erinaceus*. The ninth day at 9 a.m. the terminal part of the pricked leaf-blade had wilted and in the afternoon of the same day the wilt involved the whole leaf-blade. The following day the blades of the two leaves next above and the two next below the pricked leaf had wilted. The petioles were turgid. The eleventh day the wilt was but little if any worse than on the preceding day and the petioles were still rigid. Two days later the blades of six leaves were badly wilted or shriveled. The petioles were rigid and the terminal leaf and one or two at the base were normal. The twenty-fourth day several leaves were dead but the plant as a whole seemed to be recovering and had apparently thrown off the disease. The weather was cool. June 16 the plant had recovered and had a good color but was a trifle stunted. June 25 the plant had entirely recovered and was making a good growth.

*Remarks.*—Watermelon, *Echinocystis lobata*, and *Melothria scabra* developed only slight local signs. In one vine of *Cucumis erinaceus* only slight local signs appeared. In another vine of the same species, constitutional signs followed the local wilt, but finally the plant threw off the disease and recovered. Cucumbers first showed local signs (in the pricked parts) and then general signs ending in death.

#### INOCULATIONS OF JUNE 16, 1896

Inoculations were made in the hothouse at 11 a. m., on *Cucurbita foetidissima*, *Apodanthera undulata*, *Cucurbita palmata* (?), *Cucurbita digitata*, *Trichosanthes cucumeroides*, *Passiflora incarnata*, *Cucumis melo* var. *dudaim*, *Citrullus vulgaris*, *Echinocystis lobata*, and *Cucumis erinaceus*. All the plants were infected from culture No. 16, May 27, a tube of peptone-water (cucumber-strain). This culture would have been old and exhausted long before but for the fact that the organism made no growth in it for the first 16 days, having been kept all of this time in the ice-box at temperatures ranging from 6° to 10° C. At 4 p. m., June 12, it was removed from the ice-box and put at room-temperature (25° or 26° C.). In 48 hours it showed faint clouding and for the 36 hours preceding its use for inoculation it had been well clouded with good rolling clouds on shaking. When examined in a hanging drop the culture was seen to contain numerous rods in process of division. Some of the bacteria were actively motile, darting ahead long distances; others, slowly tumbling; others, stationary. There were a few involution forms. A copious quantity of the fluid was lifted out on a sterile platinum loop, placed on the surface of a clean leaf and pricked in with a sterile steel needle. The loop and needle were flamed after each operation and used again as soon as cold. In most instances a second loop of the culture was rubbed over the numerous delicate pricks. At the time of the inoculation the hothouse was cool, there was no wind and the sky was overcast. There could not have been a better day nor apparently a more suitable culture.

(293.) *Cucurbita foetidissima* (grown from seeds of Toumey's second sending. Looks a little different from the first sending, as if the plant were a variable one). Up to the ninth day no signs had appeared, but 2 days later (1 p.m.) there was a very slight wilt of the pricked leaf at one edge of the pricks. July 14 the plant was entirely dry-shriveled as a result of the inoculation.

(294.) *Cucurbita foetidissima*. This plant must have contracted the disease as early as the fourth or fifth day. On the sixth day the blades of five leaves were wilted, i.e., that of the pricked leaf and of two leaves above and two below. The seventh day the pricked leaf was wholly shriveled, the

petiole being still rigid. Two days later the plant was very sick, all of the leaves, seven in number, having wilted. The eleventh day the stem was still green and turgid. July 14 the plant was entirely dry-shriveled as a result of the inoculation.

(295.) *Cucurbita foetidissima* (Toumey's first sending to Mr. Hicks). The sixth day (3 p.m.) there was no sign of the disease but 23 hours later the leaf on the pricked side had changed to a dull green and was wilted over an area of about 10 sq. cm. from the pricks outward, up, down, and inward to the midrib. The rest of the plant was uninjured. The ninth day the whole of the pricked leaf had shriveled except the petiole which was turgid. For distribution of the bacteria in the petiole of such a leaf see fig. 77, and for a detail from the same see fig. 78. The wilt now also showed on the blades of three other leaves—the first down and the first two up. Two days later all but one leaf was wilted, the stem, however, was still turgid and green. On July 14 the plant was dry-shriveled, with the exception of the base of the stem which was green.

(296.) *Apodanthera undulata* (From Toumey, Tucson, Arizona). The pricked leaf was examined frequently but there was no marked result from the inoculation. On July 14 the plant was healthy. Part of the pricked area had dried out and most of the leaf-blade which was pricked was yellowish and yellow-green, but it had never shown any tendency to wilt.

(297.) *Apodanthera undulata*. The seventh day all the foliage was slowly drying out but not as a result of the inoculation. Two days later there was a slight wilt ( $3 \times 4$  mm.) in the center of the pricked part. This portion later became dried out and brown but the plant did not contract the disease and on July 14 the rest of the pricked leaf was normal.

(298.) *Apodanthera undulata*. Plant examined June 23, 25, 27, and July 3. There was no result from the inoculation. Up to July 14 the pricked leaf had not wilted and the plant was healthy.

(299.) *Cucurbita palmata*? (Seeds received from Toumey: Said to have been collected in California). Up to July 14 there had been no wilt and the plant was growing rapidly.

(300.) *Cucurbita palmata* (?). On June 25 the plant was normal. The eleventh day the pricked blade was yellow and shriveling, but 28 days after the inoculation there was still no general wilt.

(301.) *Cucurbita palmata* (?). Up to June 27 the pricked leaf showed no signs. On July 14 the pricked leaf-blade had wilted and some of those above it were yellow, but it was doubtful whether this was due to the disease because there was a fine growth of healthy vine beyond the pricked leaf and no good leaves below it.

(302.) *Cucurbita digitata* (From Toumey. The plant identified as *Cucurbita californica* looks and tastes something like this). The seventh day there were tiny dead spots in the pricked area

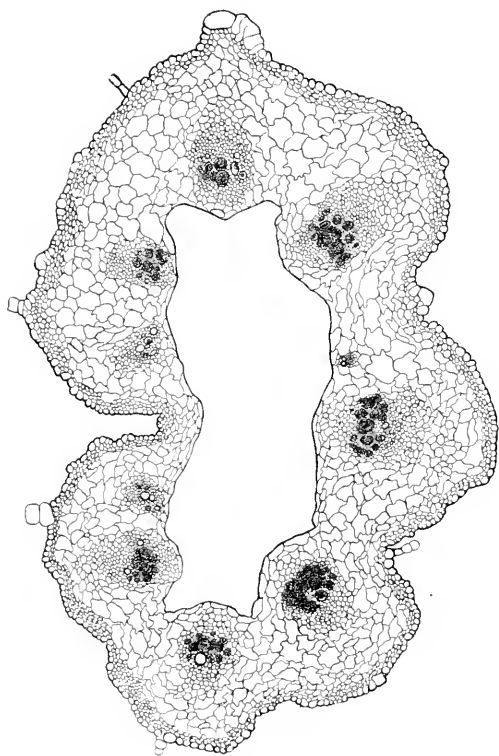


Fig. 77.\*

\*FIG. 77. —Cross-section of leaf-stalk of *Cucurbita foetidissima*, the wild gourd of the western plains of the United States, showing vascular system occupied by *Bacillus tracheiphilus*. Plant grown in a hothouse in Washington from seeds obtained in Arizona. Inoculation from a pure culture by means of needle-pricks in blade of leaf. Petiole fixed in strong alcohol, infiltrated in paraffin, sectioned on microtome, stained in carbol-fuchsin, and differentiated in 50 per cent alcohol. Drawn from section with aid of Abbe camera. Slide 254 D 1.

but 4 days later there was no wilt. On July 14 the pricked leaf was shriveled but the rest of the vine was normal.

(303.) *Cucurbita digitata*. Wilt began on the fifth or sixth day in the middle of the pricked part. The seventh day in the central part of the pricked area the tissue for 0.7 sq. cm. was dead and yellow-white. Outside of this was a narrow border of freshly wilting tissue, but five-sixths of the leaf was still normal. Two days later the whole of the pricked blade and the upper two-thirds of the petiole had shriveled. The eleventh day the second leaf was beginning to yellow and droop. On July 14 the pricked leaf and the first leaf up had shriveled. The rest of the vine was normal.

(304.) *Trichosanthes cucumeroides* (from Agr. college in Japan). The seventh day the tip of the pricked leaf was wilting but 2 days later most of the pricked leaf was normal and the wilt seemed to be dying out. The eleventh day a fresh part of the leaf had begun to wilt. On July 14 the whole of the pricked leaf had shriveled. The rest of the vine was normal.

(305.) *Trichosanthes cucumeroides*. The pricked leaf showed no signs up to June 25. The eleventh day about one-third of the apical (pricked) portion of the inoculated leaf-blade had wilted but was not yet dry. On July 14 the pricked leaf had shriveled, but the rest of the vine was normal and was growing rapidly.

(306.) *Trichosanthes cucumeroides*. There was no result from the inoculation other than local injury which did not appear until after June 27 (eleventh day). On July 14 the pricked leaf had dry-shriveled but the remainder of the vine was normal and growing rapidly.

(307.) *Passiflora incarnata*. The seventh day a whitish callous had formed around each of the pricks and there was no wilt. Up to July 14 there had been no result even in the pricked leaf.

(308.) *Passiflora incarnata*. Like the preceding. No result. There was no wilt or change of color even in the pricked leaf.

(309.) *Cucumis melo* var. *dudaim* (ripe fruit yellow and like a small round gourd; delightful odor; taste like muskmelon). The inoculation was made in an old leaf. The seventh day there was a distinct wilt in and around the pricked area. This had begun in a small way the preceding day. Two days later about one-fourth of the pricked leaf-blade was wilted. The blade of this leaf measured 3.5×3.5 inches. On the eleventh day the whole of the pricked leaf-blade was dry-shriveled. On July 14 the whole plant was dry-shriveled.

(310.) *Citrullus vulgaris*. The seventh day there was no wilt and it looked as if a cork-layer had formed around the pricks, at least there was a narrow yellow rim around each prick (estimated 0.1 mm. wide). The plant was examined June 25, 27, July 3 and 14. There was no result even in the pricked leaf. This leaf had received 67 pricks.

(311.) *Echinocystis lobata*. Wilt appeared the sixth day in the pricked portion of the inoculated leaf. The seventh day nearly the whole of the pricked leaf had wilted and 2 days later the whole pricked blade including the tip of the petiole had shriveled. This leaf was separated from the one above by an internode of 4 inches and that leaf was still normal. The first leaf below was 4 inches down and that too was unaffected by the wilt. The eleventh day the plant had developed as fine a case of the bacterial wilt as could be desired. The pricked leaf had entirely shriveled, including

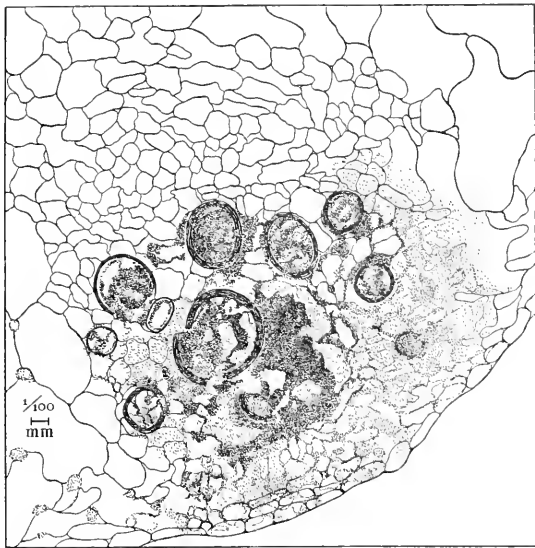


Fig. 78.\*

\*FIG. 78.—Cross-section of petiole of *Cucurbita foetidissima*, showing a bundle occupied by *Bacillus tracheiphilus* as result of a pure culture inoculation on lamina of leaf. For orientation see fig. 77, which was made, however, from another section. Drawn from slide 254 B x, with the Abbe camera. Plant No. 273

the petiole, and the wilt had extended to five leaves above (16 inches of stem) and to 3 leaves below. The stem was still green and looked normal; also the leaves farther up and lower down. On July 14 the whole plant, which was several feet long, had been shriveled for some days.

(312.) *Cucumis crinaceus*. On the ninth day there was a slight yellowing of the pricked part and of the apex of the blade beyond the pricks, but no wilt. Two days later there was very little change. July 14 the pricked leaf had dry-shriveled to the stem but the rest of the plant was normal and was making a good growth. August 15 the plant was living and growing, having overcome the disease.

(313.) *Cucumis crinaceus*. The ninth day this plant resembled the preceding. July 14 a few leaves had dry-shriveled but the bulk of the plant was uninjured and the wilt seemed to have stopped. August 15 the plant was still living and growing. It had overcome the disease.

(314.) *Cucumis crinaceus*. The seventh day only about two-thirds of the small pricked leaf had wilted and 2 days later the signs were still slight. Part of the pricked leaf-blade had dried out and the lower edges were curved in and flabby. The leaf above resembled this one in being incurved and slightly wanting in turgor. The eleventh day the wilt was still confined to the pricked leaf and the first one above, the internode between the two being very short. On July 14 the pricked leaf was dead and the tip of the branch bearing it; also some leaves lower down. Midway, however, were three good leaves. The other branch (the plant was a small one) looked healthy and it seemed at this date as if the plant would outgrow the disease. On August 15 this plant, like the two preceding, was living and growing, having overcome the disease.

*Remarks.*—The result with the watermelon confirmed the previous experiments. This plant is very resistant.

*Cucumis crinaceus*, *Apodanthera undulata*, *Cucurbita palmata* (?), *Cucurbita digitata*, and *Trichosanthes cucumeroides* were quite resistant. Some developed no signs; others only local ones; three developed constitutional signs, but recovered after a few weeks. *Passiflora incarnata* is resistant.

*Cucurbita foetidissima*, *Cucumis melo* var. *dudaim*, and *Echinocystis lobata* contracted the disease promptly and were destroyed by it.

#### INOCULATIONS OF JULY 14, 1896.

This set of experiments was made to determine whether the disease could be cut out. Twenty muskmelon plants (*Cucumis melo*) were inoculated in the hothouse from tube No. 12, July 8 (a potato culture made from a peptonized beef-bouillon culture which had been subjected to intense cold in a mixture of frozen carbon dioxide and ether). On July 11, the surface of the potato in this tube bore a good typical growth of *Bacillus tracheiphilus*. It was smooth, wet-shining, white, *i. e.*, almost exactly the color of the steamed potato, and quite sticky. All inoculations were made on the leaf-blades and as far as possible from the stem. From 30 to 60 delicate pricks were made with a steel needle sterilized in a flame and cooled each time before using. The pricked area in each case was less than 1 sq. cm. A loop of fluid from the bottom of the culture was taken out on a sterile platinum loop, placed on the clean surface of a leaf and the pricks were then made in and around this wetted surface, the drop being finally spread so as to cover all the pricks. A clean paper was then placed over the pricked leaf to screen off the bright sun.

(315 to 334.) Twenty muskmelons. Next to the lowest leaf of each plant was inoculated on the blade 3 to 5 inches from the stem.

July 31st. There has been no trace of wilt on any of these 20 plants.

*Remarks.*—All the melons were of one variety, the New Early Hackensack. My plan was to cut away each pricked leaf at its junction with the stem as soon as a trace of wilt appeared, but the entire experiment miscarried. Up to July 31, none of the 20 plants had contracted the disease. Several hypotheses occurred to me as explanations of this failure: (1) The plants were of a resistant variety; (2) The wrong organism was used; (3) The culture was dead when taken into the hothouse or, at least, that part of it in the fluid at the bottom of the tube; (4) The bacteria were destroyed by the bright sunlight in the short time which elapsed between spreading them on the leaf and pricking them in; (5) The intense heat of the hothouse destroyed them. The day was very hot and the sun bright.

Possibly the plants may have been very resistant but my opinion at the time was that the bacteria were dead when inoculated. This is possible since the tube was inoculated very copiously on the start (by means of a pipette) and would consequently convert the sugar of the potato into a harmful acid sooner than under ordinary circumstances. Probably the viscid bacteria on that part of the potato exposed to the air were still living, and very likely the experiment would have succeeded had slime been taken from the exposed surface, or had the latter been washed down into the fluid at the bottom of the tube by prolonged shaking, as in the experiment of July 16. The previous freezing had nothing to do with it, since freezing does not destroy the pathogenic properties of the organism (see experiment of July 16).

INOCULATIONS OF JULY 15, 1896.

A second set of inoculations was made in the hothouse to determine whether the disease could be cut out. The plants used were small muskmelons (*Cucumis melo*) and all the pricks were made on the apical part of the blade of the first or second leaf above the cotyledons. Many delicate pricks were made, covering an area not to exceed one square centimeter. My method of inoculation was to heat a platinum loop to redness, wait until cool, open the tube containing the culture and take out a loop of fluid from the bottom. I placed this loop of fluid on the leaf and pricked through it with a steel needle which was heated and cooled each time. The fluid was then spread so as to cover fully the pricked area in case any pricks extended outside of the liquid. The pricked portion was then covered from the direct rays of the sun for some hours. The infectious material used for these inoculations was taken from potato culture No. 12, July 8, *i. e.*, the same culture which was used for the inoculations of the preceding day.

(335 to 354.) Twenty muskmelons.  
No result.

*Remarks.*—The melons were all of one variety—Princess. It was my intention to cut away the leaves close to the stem as soon as wilt appeared, but the experiment failed, the plants being inoculated from the same part of the same tube as the preceding. It is a good illustration of the danger of putting all one's eggs into a single basket. Merely as an ordinary precaution this set ought to have been inoculated from a different culture and transfers should have been made from each one into nutrient agar just prior to the inoculations so as to know whether the bacteria were really alive. Examination in a hanging drop just prior to inoculation would also have shown whether the fluid contained motile rods suitable for inoculation. As it was, two otherwise carefully planned experiments yielded only negative and disappointing results—results which have considerable interest, however, when compared with those of the next series.

INOCULATIONS OF JULY 16, 1896.

A third set of inoculations (1<sup>h</sup> to 5<sup>h</sup> 30<sup>m</sup> p. m.) was made to see if the disease could be cut out. The plants were in a hothouse and the bacteria used were from tube 11, July 8 (a potato culture made from the bouillon culture which had been cooled to  $-77^{\circ}\text{C.}$ ). This culture was made at the same time and from the same tube as culture No. 12, July 8 (see inoculations of July 14, and 15 which failed). Loops of the liquid were also taken from the bottom of the tube but only *after it had been shaken thoroughly* in order to wash the sticky bacteria off the cylinder into the liquid. This was the only particular in which the material used for inoculation varied from that used for the preceding experiments which failed. Well-developed young, healthy, and rapidly growing cucumber plants (*Cucumis sativus*) were inoculated. The variety selected was White Wonder. Many delicate pricks (40 to 70) were made in the apical part of one leaf-blade of each plant, covering an area of not more than 1 sq. cm. The pricks themselves did the plant no injury. The platinum loop and the steel needle used in the operation were flamed and cooled each time before using.

A big loop of the fluid, containing many thousand bacteria (some of which were motile, as determined by examination under the microscope) was put on the clean surface of the leaf, spread a little and then rapidly pricked in, taking special care to make the needle-holes as small as possible. The afternoon was cloudy, rainy, and cooler than the 10 days preceding. On account of the cloudiness and moisture in the air the pricks were not covered over with papers. The plants were examined every day for the first 8 days and frequently after that. Twenty-four plants were inoculated.

(355.) This plant was 18 inches high and very thrifty. The inoculation was made on the sixth leaf 9 inches away from the stem. The pricked leaf-blade was 5 inches broad. Up to the morning of July 21 there was no trace of the disease but at 3 p.m. of the same day about 0.5 sq. cm. on one side of the pricks was wilted. The following morning there was only a very slight change. By noon of the seventh day the wilt covered about 10 sq. cm., and reached half-way down the blade. The leaf was now cut off close to the stem with a hot knife. Four days later the vine was normal, apparently, except for a droop of the first two blades below and a fainter one of the first two above the node which had borne the pricked leaf. I filled the pot several times with water but an hour later the absorption of the water had not relieved the droop of the foliage. The next day in the afternoon the first two leaves below were cut away. They had not recovered their turgor. Three days later the first leaf up was gone (removed by someone), but the blades of the next four up showed the wilt. The eighteenth day the blades of the second and fourth leaves up were shriveled but the petioles were turgid. The fourth leaf was on the same side of the stem as the second. The blade of the third leaf which was on the opposite side was flabby but had not yet shriveled. The blades of the fifth, sixth, seventh, and eighth leaves up were drooping. The others were turgid. The twenty-third day after inoculation all the leaves were shriveled and the stem itself was beginning to shrivel. The vine was about 5 feet long, *i. e.*, it trebled in length after being inoculated. It was staked up.

(356.) This was a thrifty plant about 28 inches high. The sixth leaf was pricked 10 inches from the stem. The pricked leaf-blade was 6 inches broad. The sixth day no signs had appeared but at noon of the following day there was a slight wilt in the pricked area and 2 days later this wilt covered about 5 sq. cm. The eleventh day (9 a.m., July 27) the pricked part had dried out and the wilt had increased only slightly (1 to 2 sq. cm.). At 3<sup>30</sup> p.m., however, the wilt covered an area of about 10 sq. cm. The leaf was now cut off near the stem with a hot knife. Twelve days later no further signs had appeared, but the seventeenth day after the removal of the pricked leaf (28 days after inoculation), the blade of the first leaf below and of the first and second up were flabby. Examined microscopically the petioles of these three leaves were found to contain bacteria.

(357.) This plant was 19 inches high. The inoculation was made 8.75 inches from the stem. The pricked leaf-blade was 5 inches broad. The fifth day, at 10 a.m., there had been no change in the appearance of the pricked leaf, but at 1 p.m., there was a slight wilt. At 3 p.m. I removed the leaf at the base with a hot knife, about 2 sq. cm. in and around the pricked area, having wilted distinctly. None of the other leaves ever showed any trace of the wilt. On September 23 (69 days) the plant was still living and free from the disease.

(358.) This vine was 20 inches high and thrifty. The sixth leaf-blade was 5 inches broad. It was inoculated 7.5 inches from the stem. The sixth day the pricked leaf still presented a normal appearance, but the following day at noon there were about 3 sq. cm. of wilt in and around the pricked portion. Two days later (hot again) the wilted area had increased to 5 or 6 sq. cm. The tenth day there was a bad wilt of the pricked blade, mostly without change of color, but which I could not overcome by copious watering. The next morning the leaf-blade had changed color throughout (the characteristic dull green) and the outer three-fourths of the petiole was flabby. I did not remove this leaf. The twenty-third day the blade of the first leaf down and those of the first three leaves up had wilted. The petioles were turgid.

(359.) This plant was 17 inches high and thrifty. The fifth leaf-blade which was 6.5 inches broad, was inoculated 8 inches from the stem. The fifth day, at 10 a.m. no signs had appeared but at 1 p.m. there was slight wilt in and around the pricked area. At 3 p.m. I removed the leaf with a hot knife, cutting the petiole close to the stem. About 3 sq. cm. of the leaf had wilted in the pricked area, and immediately around it. The eighteenth day (13 days after the removal of the pricked leaf), the foliage drooped a little, but it was doubtful whether this was due to the disease. The day was hot, still and cloudy. I watered the pot which had become rather dry, but this did not cause the leaves to recover their turgidity. Five days later (August 8) the blades of three additional leaves were wilted.

(360.) This plant was 19.5 inches high. The fifth leaf was inoculated 9.5 inches from the stem. The pricked leaf-blade was 5 inches broad. The eighth day there was no trace of the wilt but the



next afternoon (2 p.m.) there was wilted tissue in and around the pricked portion, covering an area of about 5 sq. cm. Two days later the wilt involved about 10 sq. cm. and reached nearly half-way to the middle of the blade. The leaf was now cut away with a hot knife close to the stem. The seventh day after the removal of the leaf (eighteenth day after inoculation), the first leaf up hung down flabby and the blades of the next three above drooped. Five days later more leaves were wilted.

(361.) This plant was 13 inches high. The sixth leaf was inoculated 7.25 inches from the stem. Its blade was 4.5 inches broad. The sixth day there were no signs of the disease but the following day at noon there was a wilt of about 3 sq. cm. in and around the pricked part. Two days later (2 p.m.) the wilt included about 8 sq. cm. and reached half-way to the base of the blade. The leaf was now cut away at its junction with the stem, using a hot knife. Eleven days later there was a distinct wilt of the leaves to either side of the pricked one. Three days later (August 8) the next two leaves farther up were wilted.

(362.) This plant was 22 inches high. The fifth leaf was inoculated 8.75 inches from the stem. Its blade was 6 inches broad. The sixth day (10 a.m.) there was no trace of the wilt, but the following noon there was wilt of about 10 sq. cm. of tissue in and around the pricked area. The wilt reached nearly half-way down the blade. The leaf was now removed close to the stem with a hot knife. Eight days later (15 days after inoculation), the blade of the first leaf up showed a decided droop. The leaves below had shriveled from other causes. The twentieth day after inoculation several more leaves above the pricked one showed the bacterial wilt and three days later there were three additional wilted leaves, farther up the stem.

(363.) This plant was 26 inches high. The sixth leaf was inoculated 10 inches from the stem. The pricked leaf-blade was 6 inches broad. The first signs of wilt were visible the ninth day (2 p.m.) and were confined to the pricked area. Two days later the pricked area was dead and the tissue around it was yellow. There had been only a slight increase of wilt, but the whole leaf had a slightly yellow look. The thirteenth day (4 p.m.) the wilted area measured about 20 to 25 sq. cm. and extended along the midrib three-fourths of the way to the petiole. The leaf was now cut away close to the stem with a hot knife. Seven days later there was a distinct wilt of several leaves above the pricked one and 3 days after that additional leaves were wilted.

(364.) This plant was 25 inches high. It was inoculated on the fifth leaf, 9.5 inches from the stem. The pricked leaf-blade was 5.5 inches broad. The plant was healthy on the afternoon of July 25. The eleventh day (10 a.m. July 27), the pricked area was dead and the surrounding tissue freshly wilted. In all there were about 3 sq. cm. of wilt. Two days later (4 p.m.) the wilted area around the pricks covered about 10 sq. cm. and was mostly dried out. The fifteenth day (2 p.m.) the wilt reached two-thirds of the distance to the petiole and covered 20 sq. cm. The leaf was now cut away with a hot knife close to the stem but 13 days later the blades of the first three leaves up were drooping. On examining each of them microscopically I found bacilli in the vessels in the base of the petioles.

(365.) This plant was 22 inches high. It was inoculated on the fifth leaf 10.5 inches from the stem. The pricked leaf-blade was 6.5 inches broad. The eighth day no signs had appeared, but the following day (2 p.m.) there were about 2 sq. cm. of wilt in and around the pricks. During the next 3 days the wilted area increased to about 10 sq. cm. The leaf was now (July 28) cut away with a hot knife at its junction with the stem. No secondary signs appeared until after August 8. On August 13 (16 days after removal of the leaf) the blades of several leaves up were drooping. I examined their petioles microscopically and found bacilli abundant in the vessels.

(366.) This plant was 18.5 inches high. The fifth leaf was inoculated 9.5 inches from the stem. The pricked leaf-blade was 6 inches broad. The fifth day at 1 p.m. there were no traces of the disease, but 2 hours later there was about 1 sq. cm. of wilt, mostly in the pricked area, but extending out a little on one side. By the next morning the wilt had increased about 1 sq. cm. and 26 hours later (noon, July 23) measured about 1.4 sq. cm., reaching half-way to the base of the blade. With a hot knife I now removed the leaf at its junction with the stem. The plant showed no constitutional signs until after July 27. Eight days after the removal of the pricked leaf (July 31) the blades of the first four leaves above were badly wilted. The first two below had dry-shriveled from other causes. Five days later five or six leaves above the pricked one had wilted and in 3 days more all the remaining leaves had succumbed to the wilt, and the vine, which was now about 5 feet long, had begun to shrivel.

(367.) This plant was 18.5 inches high. The sixth leaf was inoculated 7.5 inches from the stem. The leaf-blade was 6 inches broad. By the thirteenth day (4 p.m.) there were about 2 sq. cm. of wilt extending along one side of the pricked area most of which was still sound. From this I inferred that the infection resulted from a few bacteria lodged on one side of the pricked area. Two days later (July 31, 2 p.m.) there were about 10 sq. cm. of wilt reaching more than half-way to the base

of the blade. The leaf was now cut away close to the stem with a hot knife. There were no signs until after August 5. On August 8 (eight days after the removal of the pricked leaf) the blade of the first leaf up hung flabby and the one below had wholly shriveled. The next three up showed a slight droop. Five days later several more leaves were drooping.

(368.) This plant was 20.5 inches high. The sixth leaf was inoculated, 6.5 inches from the stem. Its blade was 5.5 inches broad. At 9 a.m. of the fourth day there were no signs, but at 2 p.m. there was a wilted area extending from the pricked part toward the tip of the leaf, affecting about 2 sq. cm. At 5 p.m. the wilt was decided, involving all of the pricked area and a narrow strip extending nearly to the apex of the leaf (about 2 cm.). The pricked leaf was now cut away at the base. This plant was examined July 27 and 31, August 8, 13, 17, 19, 28, and at later dates. None of the other leaves became affected. On September 23 the plant was still living and free from this disease.

(369.) This plant was 16 inches high. The fifth leaf was pricked 9 inches from the stem. The pricked leaf-blade was 6 inches broad. The fifth day at 10 a.m. the leaf was still normal in appearance but 3 hours later there was a slight wilt in and around the pricked area. By 3 p.m. the wilt had spread rapidly. It then covered about 8 or 9 sq. cm. and reached nearly half-way down the blade. I now removed the leaf at the base using a hot knife. (This leaf was saved in alcohol for sections.) There were no signs until after July 27. Ten days after the removal of the pricked leaf (July 31) the blades of the first two leaves above were drooping badly. The first leaf below was normal. Five days later several more leaves above the pricked one were wilted. The eighteenth day after the removal of the pricked leaf additional leaves near the top of the vine were wilting.

(370.) This plant was 19 inches high. The fifth leaf was inoculated 7.75 inches from the stem. The pricked leaf-blade was 6 inches broad. The fifth day, at 10 a.m. there were no signs but at 3 p.m. there was wilt of about 0.5 sq. cm., in the center of the pricked area. The wilt increased very little over night but the seventh day at noon it covered about 5 sq. cm. around the pricks. The eighth day was cool with heavy rains in the afternoon and the wilt was at a standstill. The following day, however, was sunny, transpiration was greater, and the wilt of the pricked leaf, at 2 p.m. covered about 12 sq. cm. and reached nearly half-way to the base of the blade. I now cut the leaf away at the stem with a hot knife. None of the other leaves contracted the disease.

(371.) This plant was 20 inches high. The fifth leaf was inoculated 9.5 inches from the stem. The pricked leaf-blade was 5.5 inches broad. There were no signs up to noon of July 23. The ninth day (July 25, 2 p.m.) there was wilt of about 3 sq. cm. in and around the pricks. Two days later (10 a.m.) the pricked area was dead but there had been only a slight increase of wilt. The following afternoon (July 28, 5 p.m.) the leaf was cut away close to the stem with a hot knife. At that time there were about 8 sq. cm. of wilted tissue in the vicinity of the pricks. There were no constitutional signs until after July 31. On August 8 (11 days after the removal of the pricked leaf) the first leaf below was shriveled and the blades of the second below and first above drooped a little. The sixteenth day after the removal of the pricked leaf there was bad wilt of several additional leaves above the inoculated one. I now cut off three leaves and examined them. On touching the cut ends with my finger the bacteria in the vessels strung out 1 to 2 cm. in numerous fine, sticky, cobwebby threads.

(372.) This plant was 22 inches high. The fifth leaf was inoculated 9.5 inches from the stem. The pricked leaf-blade was 6 inches broad. Up to the fifth day at 3 p.m. no signs had appeared, but the morning of the sixth day about 1 sq. cm. of tissue in the pricked area was wilted. By noon of the following day the wilted area was about fifteen times as large and reached half-way to the base of the blade. The leaf was now cut off at its junction with the stem, using a hot knife. None of the other leaves contracted the disease. On September 23 (60 days) the plant was still living and free from the disease.

(373.) This plant was 23 inches high and very thrifty. The sixth leaf was selected for inoculation. Its blade was 5 inches broad, and the pricks were made 8 inches from the stem. On the seventh day at noon there was about 1 sq. cm. of wilt in the outer part of the pricked area. During the next 2 days the wilted area increased not more than 1 sq. cm. The eleventh day, at 10 a.m., the pricked area was dead and the surrounding tissue yellow, but there was only a slight increase of the wilt. Four days later (July 31, 2 p.m.) there were about 25 sq. cm. of wilt, reaching three-fourths of the way to the base of the blade. With a hot knife the leaf was now cut away at its junction with the stem. There were no constitutional signs until after August 5. Eight days after the removal of this leaf the blade of the first leaf up was drooping decidedly and the blades of the next two above showed a faint wilt (August 8, 10 a.m.). I now cut away the petiole of the first leaf up at its base, using a hot knife and examined it by touching the cut end with my finger. The surface ooze was sticky and stringy a little. During the next 5 days several additional leaves wilted.

(374.) This plant was 22 inches high. It was inoculated on the fifth leaf 10.5 inches from the stem. The pricked leaf-blade was 6 inches broad. Up to the fifth day at 10 a.m. there were no signs, but 3 hours later there was a slight wilt, and at 3 p.m. there was a decided wilt involving about

3 sq. cm. This began in the pricked area and extended outward to the apex of the leaf. The leaf was now cut off at the base of the petiole with a hot knife. None of the other leaves wilted as a result of the inoculation. The basal leaves shriveled the fifteenth day, but from age, not from the wilt. September 23 the plant was dry but bore a healthy green fruit. The diseased plants had been bone-dry for weeks.

(375.) This plant was 16.5 inches high and thrifty. The inoculation was made on the fifth leaf 8 inches from the stem. The pricked leaf-blade was 6.5 inches broad. Up to the fifth day at 10 a. m. no signs had appeared. Three hours later there was a slight wilt, and at 3 p. m. this involved about 1 sq. cm. The wilt was on one side of the pricked area, and extended toward the margin of the leaf. The next morning the wilted area did not exceed 2 sq. cm. Twenty-six hours later (July 23) there were about 10 sq. cm. of wilt. I now cut off the leaf, with a hot knife, at its junction with the stem. There were no constitutional signs until after July 27, but 8 days after the removal of the leaf (July 31) the blade of the first leaf below and the first above drooped very decidedly. Between this date and August 5 several of the leaf-blades farther up had wilted. On August 8 (the sixteenth day after the removal of the pricked leaf) three more leaves above the inoculated one were wilted.

(376.) This plant was 22 inches high. The fifth leaf was inoculated 10.25 inches from the stem. The pricked leaf-blade was 5.5 inches broad. The seventh day after the inoculation (July 23, noon) there were about 3 sq. cm. of wilt in and around the pricks. Two days later there was a wilted area of about 15 sq. cm. extending a little over half-way to the base of the blade. I now cut away the leaf close to the stem using a hot knife. There were no constitutional signs until after July 27. Six days after the removal of the pricked leaf (July 31) the blade of the first leaf below and of the first two leaves above drooped very decidedly. The eleventh day after the removal of the pricked leaf, I cut away five leaves with wilted blades, all above the inoculated one. Three days later (August 8) two more leaves above showed a decided droop of the blades.

(377.) This plant was 14 inches high. The fifth leaf was pricked 7.75 inches from the stem. The pricked leaf-blade was 5.5 inches broad. The first wilt was noted the seventh day in and around the pricks. It then covered an area of about 2 sq. cm. Two days later (2 p. m.) about 7 sq. cm. of tissue had wilted. The eleventh day (July 27) about 12 sq. cm. of tissue extending about one-third of the distance to the base of the blade had wilted and changed color. The pricked area and the tissue immediately around it were now dead. The leaf was cut off close to the stem with a hot knife. The plant showed no constitutional signs until after August 5. Twelve days after the removal of the pricked leaf the first leaf below was shriveled, probably from age, and the first leaf up showed a decided droop of the blade due to the disease, I cut the petiole and saw the bacterial slime string out. The next two leaves up showed a slight droop of the blades. Five days later (August 13) two more leaves above the pricked one were wilting.

(378.) This plant was 22 inches high. The fifth leaf was pricked 10 inches from the stem. The pricked leaf-blade was 5.5 inches broad. The first wilt was noted at noon of the seventh day. It then covered about 1 sq. cm. of the pricked portion. Two days later (July 25, 2 p. m.) the wilt involved about 10 sq. cm. of leaf surface and reached nearly half-way to the base of the blade. I now cut away the leaf at its junction with the stem, using a hot knife. There were no constitutional signs until after July 27. Six days after the removal of this leaf (July 31) the blades of the first five leaves up were drooping very decidedly. The leaves below had shriveled from age. Twenty days after inoculation I cut away six petioles of wilted leaves to put into alcohol. The twenty-third day August 8 one more leaf up showed wilt of the blade. The stem was still green and turgid.

*Remarks.*—This experiment is in striking contrast to those of July 14 and 15. Everyone of the twenty-four plants contracted the disease, and in each case it first appeared in the pricked area. Nineteen of the plants subsequently developed constitutional signs and died of the disease. No general signs appeared in the other five plants (Nos. 357, 368, 370, 372 and 374), *i. e.*, the disease was stopped by the removal of the affected leaf. In eighteen cases the amputation of the affected leaf did not check the spread of the disease, but it is apparent that a prompt removal of the pricked leaves, to wit, on the day the signs first appeared, would have considerably increased the number of recoveries. This is deducible from the fact that in those which did escape, the amputations were performed very promptly. It is not likely, however, that this method of treatment will ever be recommended for general use, since, in most cases, the wilt of the leaves would not be detected in time.

The experiment was practically closed the forty-third day after inoculation (August 28) but the plants stood on the bench in the hot-house until September 23. On that date all of the diseased plants were bone-dry and had been so for several weeks. Three of the plants

which did not develop secondary signs were still living (Nos. 357, 368 and 372); one was dry (374) but bore a green healthy fruit, and of the fifth (370) no record was made later than August 28. The first case (No. 368) appeared on July 20, at 2 p.m., *i. e.*, at the end of the fourth day. Eight cases developed at the end of the fifth day (Nos. 355, 357, 359, 366, 369, 370, 374 and 375). One case (372) appeared after about 5½ days. Eight cases developed at the end of the seventh day (Nos. 356, 358, 361, 362, 373, 376, 377 and 378). Four cases appeared at the end of the ninth day (Nos. 360, 363, 365 and 371), and one plant (364) came down the tenth or eleventh day. The last plant to become diseased was No. 367. This did not show any signs until the thirteenth day. With the exception of the stems of 355 and 366, which were beginning to shrivel, all of the stems were turgid and sound externally until after August 8. Throughout, the plants were free from insects.

The plants were otherwise very healthy, were watered regularly and properly cared for, and the signs obtained were characteristic of the disease. They could be ascribed only to the initial bacterial infection of July 16. In many cases the removal of the inoculated leaf was delayed so long after the first signs appeared (several days) that we can not tell therefrom how short a number of days is requisite for the general infection of the plant when the bacteria are introduced into the blades of the leaves. A few, however, give us some basis for judgment. In vine 355, at the end of 7 days, the bacteria were 7 inches in advance of the signs of wilt, *i. e.*, they had already traversed the vessels a distance of 9 inches from the point of inoculation (how much farther we can not tell) and had entered the stem, as shown by the subsequent behavior of the plant. In 356, at the end of 11 days, the bacteria were at least 9 inches in advance of the signs of wilt and had passed through the spiral vessels of the leaf a distance of 10 inches (or more) into the stem, as shown by the subsequent behavior of the plant. In 359, at the end of 5 days, as shown by subsequent signs, the bacteria had entered the stem, having passed through 8 inches of vascular system and being that much at least in advance of the signs of wilt. In 369, in 5 days, as shown by subsequent signs, the bacteria passed through 9 inches of tissue (vascular system) and entered the stem. In 372, on the contrary, at the end of 7 days, the bacteria had not yet entered the stem (9.5 inches distant), although at the time the leaf was cut away there were 15 sq. cm. of wilted tissue, reaching nearly to the middle of the blade, and signs had been present for at least 26 hours. From these results we may conclude that under favorable circumstances infection of the main axis in the cucumber is comparatively prompt, the bacteria being able to pass down through the vessels of the leaf at the rate of about 0.75-inch to 2 inches (2 to 5 cm.) a day.

Weather conditions have much to do with the rate of progress of this disease. Cool weather retards it, warm weather hastens it, extremely hot weather if long continued checks it altogether.

Following these inoculations, daily weather records do not appear to have been kept, at least on the sheets bearing my pathological memoranda, but there are occasional references to the weather. It was cool on July 23, and cool with heavy rains on July 24. This weather temporarily checked the progress of the disease. It was hot on July 25, and on July 29 and 30. It was cooler on July 31, but windy so that transpiration would be rapid. It was very hot on August 5 to 10. On the unrecorded days there was probably the ordinary summer weather.

Between August 5 and 8 numerous freshly wilting leaves (secondary wilt) were cut from these plants and fixed in strong alcohol to determine whether the bacteria are actually in the vessels of the leaf at the time the secondary wilt appears or whether this wilt is due simply to the plugging of the vessels of the stem. These leaves were well grown and sound externally. Thin microtome sections were made from the basal part of the petiole of 66 of these leaves after infiltration with paraffin. These sections were fixed on clean slides and carefully examined after removal of the paraffin and staining in carbol fuchsin. Bacteria

can not be demonstrated in every one but they occur in 61 of them; no fungi are present, neither are there any insect-injuries. In most cases the bacteria are confined strictly to the spiral vessels of these petioles and they do not occur in all of these, nor in all of the bundles. They are not present in the phloem, the cortical parenchyma or the tissues between the bundles. Summarized, the amount of bacterial infection in the basal part of these petioles is as follows: (1) In a few petioles nearly every bundle is occupied and bacteria occur in many vessels, with cavities in two or three cases; (2) in 5 no bacteria detected; (3) in by far the greater number the bacteria are confined to a few vessels of a few bundles. In groups 2 and 3 the wilt can be accounted for only by bacterial occlusions lower down *in the stem itself*.

From this experiment we may also conclude that White Wonder is a very susceptible variety and one to be rejected in regions much subject to this disease.

This single experiment, purposely given in much detail, is sufficient, in my judgment to establish not only the bacterial nature of this disease but also the general movement of the bacteria through the spiral vessels of the plant.

#### INOCULATIONS OF JULY 23, 1896.

Another attempt was made in the hothouse to transfer the disease to plants by means of the beetle *Diabrotica vittata*. The vines used were cucumbers and muskmelons (Nos. 379-429). I had grown them from the seed and transplanted them some weeks before into benches filled with good earth. They occupied the whole of a small greenhouse. They had been well watered and the temperature had been high (hot July weather). As a result the growth had been rapid and at the time of inoculation the vines presented a very thrifty appearance. There were about an equal number of each. The cucumbers were from 4 to 6 feet high with hundreds of leaves, some of which were 9 inches broad. There was not a yellow, dwarfed, or fungus-spotted leaf in the whole house and the plants had been remarkably free from aphides. No water had been put on the foliage. The muskmelons were equally healthy but were younger plants and had not made as much growth. When ready to make the inoculations I collected fifty to a hundred specimens of the striped cucumber-beetle (*Diabrotica vittata*) from squash-vines in Mr. Curtis's field south of Anacostia, where no wilt had yet appeared. Most of them were taken from the interior of squash flowers where they were in hiding through the day. These were colonized (July 23, p.m.) on five cucumber-leaves cut from plants infected July 16 (third set of inoculations to see if the disease could be cut out). Each of these leaves contained from 10 to 15 sq. cm. of freshly wilted leaf surface. Only the petioles, the wilted part of the blades and a narrow border of the blade surrounding the wilt was put in, so that the beetles would be compelled to feed on diseased tissues or else attack the hard petioles. These beetles feed mostly by night or in the early morning. At 5<sup>h</sup> 30<sup>m</sup> the next morning the beetles had riddled the wilted parts with holes. They seemed to have fed exclusively on the parenchyma of the blade and must have consumed enormous quantities of living bacteria. The mouth parts of every one must have been infected. They were now turned loose on the cucumber and melon vines and began to feed at once. The day was cool and rainy. Several additional colonizations were made since it was believed that there were many chances for failure. Such additional colonizations were made on July 24, 3 p.m., July 26 (12 beetles), July 28 (7 beetles), July 29 (150 beetles fed 12 hours on freshly wilted leaves and turned loose at 5 a.m., after the leaves were riddled with holes), August 1 (20 beetles), August 4 (200 beetles fed over night on freshly wilted cucumber leaves and set free at 5<sup>h</sup> 30<sup>m</sup> a.m., when the leaves were riddled with gnawings). The beetles were allowed to feed on diseased material from 10 to 19 hours and were then turned loose in the greenhouse.

On August 4 (5<sup>h</sup> 30<sup>m</sup> p.m.) there had been signs of the wilt for some days but inasmuch as the plants had been sprinkled with fine tobacco dust for aphides I thought possibly the injury was due to that and I neglected to examine any of the wilting leaves for bacteria (a serious omission).

On September 22 the experiment was closed. It failed, apparently because of the extreme heat. The plants were examined frequently but I could find no secondary wilt. August 10 was the hottest of 4 very hot days. On that day it was 37° C. for some hours and might have been considerably hotter in this small house for a short time, possibly as hot as 43° C. (the thermal death point of the organism). Thermometers about town registered 98° to 103° F. and it would of course be warmer in the glass house exposed to the sun. In this connection see thermal tests on p. 293. If I were to repeat the experiment I would liberate the beetles at midnight and divide the house, keeping one part cool.

#### FIELD OBSERVATIONS ON AUGUST 3, 1896.

On July 22, 1896, at Mr. Curtis's place 7 miles southeast of Washington, I tried to show Mr. Henry G. Hubbard, the entomologist, my wilt disease and failed. Mr. Curtis had about an acre of pumpkins and squashes of various ages, some covering the ground and in full bloom, others not yet in bloom and only just commencing to "run." There were at that time a very few wilted plants which Mr. Curtis attributed to borers and I to the bacterial wilt but as I could not find sticky slime inside the stems and had no microscope with me my diagnosis was unsatisfactory. The field was revisited 12 days later (August 3). There were then numerous well-developed cases of the bacterial wilt—long shoots with all the leaves drooping, and more interesting still, there were dozens of big vines which showed no general wilt, but had single leaves, usually toward the base of a stem, which bore characteristic wilt patches, *i. e.*, pale-green flabby spots varying from a few square inches to areas as big as the palm of my hand. In all such cases this wilt appeared to have spread from gnawed places.\* Moreover I saw the striped cucumber-beetle (*Diabrotica vittata*) eating holes in such wilted spots exactly as if this part of the leaf were a little tenderer or otherwise more desirable food (see page 215). There were on the vines great numbers of this beetle. A few specimens of *Diabrotica duodecimpunctata*, and a very few of *Coreus tristis* were observed.

My prediction that 90 per cent of Mr. Curtis's squash-vines, especially the older ones, would have the wilt by September came true. They were planted on the ground used for squashes 2 years before. Then nearly the whole field contracted the disease although the vines looked well at a date about corresponding to this time. The chief mischief was being done by *Diabrotica vittata*.

This insect feeds mostly in the evening, night, and early morning. In the middle of the day it hides away from the hot sun. It specially likes to take shelter inside squash and pumpkin flowers which have recently opened. The older flowers are not to its taste.

*Query:* Why can not squash flowers be used as traps for this insect? Hand picking of the staminate flowers in the middle of the day when they inclose from one to a dozen of the beetles would greatly reduce the numbers of this pest and, in conjunction with removal of wilted vines as fast as they appear, would go far toward checking the spread of the wilt disease.

This disease may certainly be controlled by destroying the insects which distribute it and to that end their habits should be studied more carefully.

#### INOCULATIONS OF JULY 26, 1897.

Four cucumber-vines (*Cucumis sativus*) and two vines of a cucurbitaceous plant from Mexico were inoculated in the hothouse with bacteria from a well-clouded tube of beef-broth (tube 2, July 19) made directly from the sticky interior of a diseased cucumber-stem from Virginia (near Norfolk). No statement as to the size or age of the plants. The inoculations were made by means of numerous needle-pricks on two leaves of each vine. There

\*In nearly a thousand cucumber leaves examined in July, 1893, on this same farm, for very early stages of the wilt, the gnawings of *Diabroticas* occurred in the diseased areas and seemed to have preceded the appearance of the wilt, *i. e.*, the gnawed part was dried out as if older than the rest of the wilt. (See plate 1, fig. 1.)

were no signs on any of the plants until after July 30. The Mexican plant is Dr. Edward Palmer's No. 1801a, and was grown from seeds of his collecting.

(430.) Cucumber. Up to August 2 (seventh day) there was no result from the inoculation.

(431.) Cucumber. The fifth day there was a distinct wilt of several square centimeters around the pricks on each leaf.

(432.) Cucumber. The fifth day there was a distinct wilt of one square centimeter in the pricked area on one leaf while the whole blade of the other leaf was wilted and collapsing.

(433.) Cucumber. The fifth day there were several square centimeters of wilt in the pricked area on both leaves.

(434.) Cucurbitaceous plant (collected in Mexico). No result by the sixth day. No further record.

(435.) Duplicate of 434. The sixth day both leaves were normal. No further record.

*Remarks.*—Three out of the four cucumbers contracted the disease promptly. The ill-scented Mexican plant bore yellow flowers; long, warty fruits, and leaves suggestive of *Momordica*.

#### INOCULATIONS OF AUGUST 21, 1897.

A series of inoculations was made on watermelon vines (*Citrullus vulgaris*) in a garden at Hubbardston, Michigan. The bacteria were taken from a potato-culture of *B. tracheiphilus* (tube 2, July 23) which had been re-inoculated July 26 with 0.2 cc. out of tube 1, July 19. This potato-culture was very sticky and in fine condition on August 12 when it was exhibited at the Detroit meeting of the American Association for the Advancement of Science, but at the time of the inoculations it had been considerably exposed to the light and was past its prime, although I believed it to be alive. It was not, however, tested under the microscope or by transfer to other media as it would have been had I had laboratory facilities. The inoculations were made in the ordinary way, *i. e.*, by means of a dozen or two needle-punctures on the leaf-blade and within an area of 2 or 3 sq. cm. The extreme upper part of the potato bore the stickiest slime and this was used for most of the inoculations, but some were made in the following way: Numerous punctures were made and then the tube was tilted until the cotton plug was wetted. This plug was then taken and mopped over the pricked area. The fluid in the bottom of this tube was very milky. The inoculations were made at sunset so as to avoid the immediate evil effect of light. As a check on the virulence of the culture eight muskmelon leaves belonging to five plants were inoculated in the same way. On four leaves the bacteria were pricked in; on the others they were mopped in after the pricks were made.

(436-439.) Watermelons. Sixteen healthy leaves belonging to four vines were inoculated, eight in the ordinary way, four by the second method described.

There was no result.

(440-444.) Muskmelons. Eight leaves belonging to five plants were inoculated, four by the second method.

The inoculations failed.

*Remarks.*—The observations were continued until September 12. The weather was very dry. Probably the potato culture was dead. It will be remembered that it was 26 days old and that it had been very copiously inoculated on the start.

#### INOCULATIONS OF JULY 11, 1898.

A series of inoculations was made on the wild bur cucumber (*Sicyos angulatus*) growing in a garden at Anacostia, D. C. The plants were large and covered the ground, the leaves being 4 to 8 inches broad, and all were perfectly healthy. All of the inoculations were made by pricking in the bacteria (cucumber-strain) with a sharp steel needle.

(445-456.) About a dozen leaves belonging to several different plants were inoculated in the blades from a pure agar-culture (tube 3, June 30, re-inoculated July 7.) The seventh day there were seven beautifully typical cases in as many of the pricked leaves. These were in all stages from one just beginning to change color and wilt around the punctures to one wholly collapsed. There was

now an enormous tangle of foliage and all the inoculated leaves which I could find were the seven diseased ones. Signs appeared only in the pricked leaves. Several of these leaves were being gnawed by the striped cucumber-beetle (*Diabrotica vittata*) but almost exclusively in the wilted, softened parts. A week later the disease was progressing typically. The blade of the leaf which showed only a tiny wilted area around the pricks on July 18 was now two-thirds wilted. Several of the inoculated plants now showed constitutional signs, *i. e.*, other leaves up and down the stem were flabby or wholly collapsed. One of these plants was brought in and examined under the microscope, bacteria being found in the vessels. The bacteria also strung out one centimeter when the sticky cut surface was touched with the finger. The plant was saved in alcohol. The disease had also been transmitted to at least one healthy vine (one leaf) by the bites of the *Diabrotica*. This beetle was observed feeding on the wilting leaves the week before and also to a slight extent, on the sound ones and one of the latter now showed several square centimeters of wilt around the bitten part.

(457-477.) About twenty leaf-blades belonging to several different plants were inoculated directly from muskmelon petioles showing the sticky bacterial exudate. There was no definite result until after the seventh day. On July 25 several plants showed typical signs in the pricked leaves.

*Remarks.*—This experiment adds another plant to the list of possible hosts. These observations also tend to confirm the belief already expressed that the striped cucumber-beetle *prefers the wilted leaves* and is consequently admirably adapted to spread this disease.

The foregoing experiments were all made by the writer. Numerous additional ones, partly by the writer and partly by his assistants (plate 14) have been made since this date, but need not be mentioned here since they are not contradictory. Some of them have already been referred to in the first part of the discussion of Etiology, where also are some important observations on the distribution of the disease by *Diabrotica vittata*.

Mention should be made, however, of some watermelon plants inoculated November 17, 1903. Of these, two plants (No. 530, variety Phinney's Early, and No. 534, variety, Mountain Sweet) showed wilt of the inoculated leaf and were brought in and put into alcohol on December 9 and 10.

Nos. 526 and 528 (variety, Triumph), inoculated at the same time, showed secondary wilt on December 5 and December 7, respectively. These also were preserved in alcohol. Sections from the stems of the ones last mentioned show the presence of bacteria in all of the vascular bundles. An attempt to plate out the organism miscarried.

From the foregoing inoculations we may conclude that in wilting cucumbers the organism present in the tissues is sometimes *B. tracheiphilus* and sometimes *B. tracheiphilus* f. *cucumis*, whereas in squashes it is always or nearly always the first and more virulent strain. This being true we might then expect some isolations from cucumbers to be infectious to squash and others not, whereas all isolations from squash should infect both cucumbers and muskmelons.

I can not say whether there are any tangible cultural differences. In one test in litmus milk at the end of two weeks the squash strain looked exactly like the check tubes, while the cucumber strain was slightly darker. I cultivated out both on potato in typical form.

#### EFFECT OF WATER ON CUCUMBER-WILT.

Experiments in the greenhouse in May, 1895, showed that when water is withheld or given sparingly the bacterial wilt becomes visible sooner than when the plants have an abundance. If water be given to such diseased plants in abundance the leaves least wilted will frequently become turgid again for a few hours. The same thing occurs in the field, especially with squashes.

#### VARIETIES ATTACKED.

The writer has observed this disease or received reports of its occurrence in the following varieties of cucumbers: White Wonder, White Spine, Long Green Japanese, Long Green, Fordhook pickling, Telegraph, Large English (few seeded sort).



It has been observed or reported in the following varieties of muskmelons: Early Hackensack, Shumway's Giant, Dudaim, Rocky Ford.

It has been observed in the following varieties of squash: Hubbard, yellow Crookneck, Long Island White Bush, Early Yellow Bush Scallop, White Summer Crookneck, Boston Marrow.

July 14, 1909, the disease was observed near Washington on Venetian squashes, grown from imported seed.

#### MORBID ANATOMY.

There are no hyperplasias in connection with this disease. It is principally a disease of the spiral and ring-vessels, and their entourage in the stem of the host. These vessels are arranged in a group toward the inner part of each bundle. They are embedded in a mass of thin-walled living parenchyma, which is separated from the inner phloem by a thin band of tissue somewhat resembling cambium in structure, and sometimes called pseudocambium. By its outer face, the tissue containing the spirals and ring-vessels joins on to the lignified tissue or xylem proper which contains the large pitted vessels embedded in pitted, lignified connective tissue. The spiral-vessels and ring-vessels are always the first part of the stem to be occupied by this bacillus. The reason for this is not far to seek. It lies in the fact that they are the only part of the xylem-portion of the bundle which passes out from the stem into the leaves to form the xylem-part of the veins of the leaf. Since the infections are entirely through the leaf-surface (so far as we yet know\*) and since the organism passes downward into the stem exclusively by way of the spiral-vessels of the petiole, it is at once apparent why the spiral-vessels of the stem are the first part of that organ to be invaded. In the stems of the cucurbits subject to this disease there are nine or ten separate vascular bundles and, consequently, on cross-section there are, or may be, nine or ten distinct bacterial foci corresponding to as many groups of spiral-vessels and ring-vessels (figs. 67, 77, 78, 80). The organism always appears in these spiral-vessels in enormous numbers, soon filling them completely (fig. 79). The next stage in the progress of the disease is the destruction of the walls of these vessels. This appears to take place by solution, but perhaps also by rupture, these walls consisting of a very thin non-lignified membrane the only lignified parts being the spiral-thickenings or ring-thickenings. The bacteria now invade in great numbers the thin-walled living parenchyma surrounding these

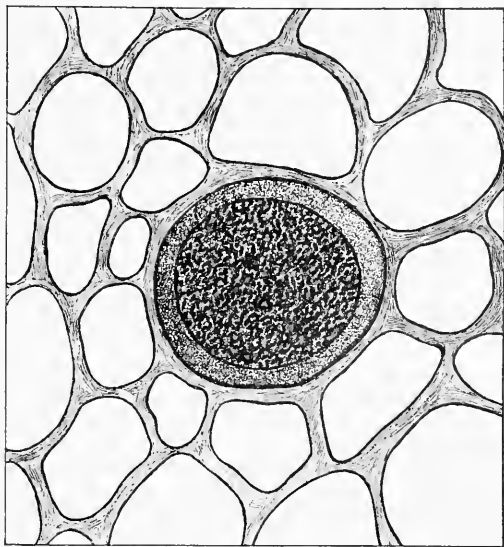


Fig. 79.†

\*Further studies should be made to determine whether infection may not also take place through the root-system.

†Fig. 79.—Cross-section of a cucumber stem from field, showing a small spiral vessel filled with *Bacillus tracheiphilus*; contents of non-parasitized vessel-parenchyma-cells omitted. Drawn from a photomicrograph of a thick, unstained glycerin mount made in 1893 (the year I discovered the disease). x 1000.

vessels. The cells are separated from each other, crushed and dissolved (?) their place being taken by the rapidly multiplying bacillus (fig. 62). For an earlier stage of bundle infection see vol. 1, fig. 9. In this way cavities arise which by fusion with other cavities lead to the honey-combing and more or less complete destruction of this part of the bundle and consequently to the interruption of its function, *viz.*, the movement of water. The bacteria also pass outward through the lignified tissues (by way apparently of the pits) into the large pitted vessels, several to many of which are often filled partly or completely before there is any destruction of the phloem or of the general connective tissue of the stem. In the end, the bacteria may be found also in the phloem and outside of the bundles in the surrounding tissues. For an especially good example of a late stage in which the bacteria have passed beyond the limits of the bundle and may be seen occupying the intercellular spaces and the interior of parenchyma cells see fig. 81. By this time, however, the stem begins to shrivel from loss of water, and the activities of the organism cease, so that the phloem and the tissues lying between the bundles, or beyond them toward the periphery of the stem, are seldom occupied to any great extent. Frequently pitted vessels at the outer

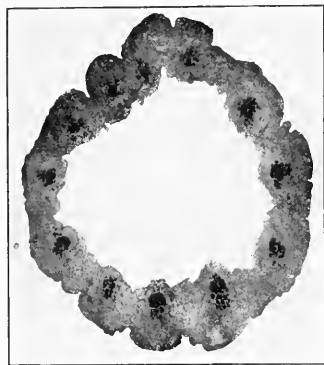


Fig. 80.\*

angles of the xylem become filled in advance of those in the middle. The lignified tissues are not dissolved, but the thin non-lignified membrane separating the pits on contiguous vessels must be ruptured or dissolved by the bacteria—otherwise it is impossible to account for their diffusion into the connective tissue of the xylem and from one pitted vessel to another.

It is very easy to demonstrate microscopically the presence of the bacteria in the vessels, to cultivate them therefrom (when the right methods are used) and by means of sections made from pieces embedded in paraffin to show all stages in the destruction of cells and in the formation of cavities in the bundles. The organism occurs also in the green fruits of cucumbers and produces therein the same occlusion of vessels and breaking down of neighboring cells, with the formation of small bacterial cavities, as in the stem. The fruit finally shrivels and the flesh sometimes has a water-

soaked look about the bundles, but there is no general disintegration of the parenchymatic tissues, *i.e.*, no soft rot.

Numerous examinations under the microscope have disclosed no tendency of the cells of the host-plant to enlarge or divide in the presence of the organism, nor have I detected any distortions or suppressions of particular systems of tissues such as we commonly find in certain other bacterial diseases. The tissues of the attacked plant seem unable to react, except that, as already mentioned, I have observed in the field, in certain squashes attacked by this organism, certain proliferations which, rightly or wrongly, I have attributed to its presence in the tissues; and also in certain inoculated squash-cotyledons a suggestion of cork-formation in the pricked area, and a very slow multiplication of the bacteria in the bundles.

#### THE PARASITE.

*Bacillus tracheiphilus* EFS.—The cause of this disease is a short, straight rod with rounded ends (figs. 57 and 82). When growing rapidly in the plant or on culture-media it commonly measures 1.2 to 2.5  $\mu$  by 0.5 to 0.7  $\mu$ , but it may be longer or shorter or thicker or

\*FIG. 80.—Cross-section of a squash petiole, showing 12 vascular bundles occupied and destroyed by *Bacillus tracheiphilus*. Tissue between bundles and toward surface is free from bacteria. Inoculation was made Aug. 10, 1905 (Colony E, House 4), on blade of leaf by needle-pricks. From a photomicrograph. Slide 354-3. For detail see fig. 81.

thinner, according to age, culture-medium, and kind of stain used. Flagella-stains in particular, affect an outer part not stained by ordinary methods (figs. 83 and 84). The organism has a distinct capsular portion, the solution of which appears to give rise to the viscosity. This viscosity occurs during active growth, and may continue for some time. When taken directly from the plant (fig. 52) this bacillus is usually viscid but not always. Often with care the slime may be stretched out to the distance of 20 to 40 cm. (once 76 cm.). The resulting cobwebby threads generally yield pure cultures of this organism and when stained on a slide and examined under a microscope are seen to be made up of bacilli embedded in a tenuous slime which separates them by considerable intervals (Vol. I, figs. 13 and 14). The

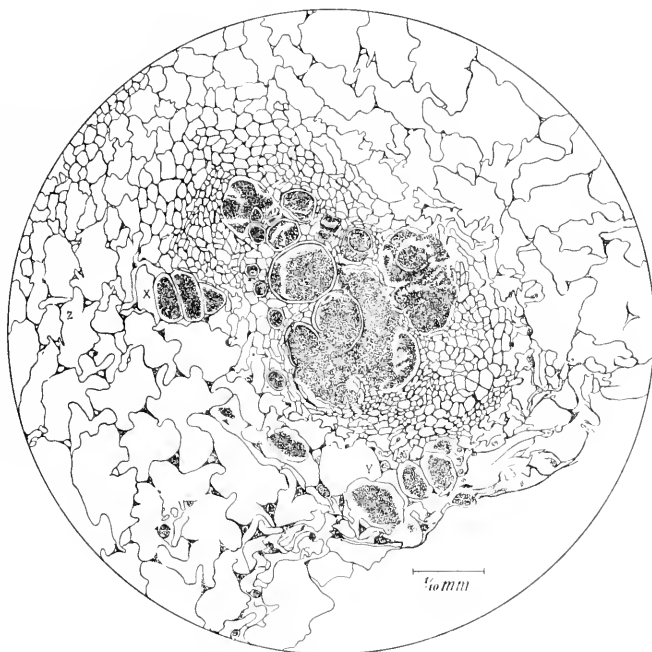


Fig. 81.\*

organism is also sticky, at least in some of the stages of its growth, on agar, gelatin, potato, carrot, sweet potato, coconut-flesh and various other solid culture-media. In one instance the slime from a potato-culture was drawn out 53 cm. before it gave way. On potato, up to the sixth day and sometimes longer, the organism is actively motile, even when examined from very viscid cultures. Potato cultures 10 to 18 days old are usually as sticky as younger ones. This slime does not dissolve readily in water and hence failure may occur in making poured plates. Cultures in potato-broth and in sugared fluids finally become ropy, and then most of the individuals or all of them are dead.

\*Fig. 81.—Cross-section of one bundle of fig. 80. At bottom and left-hand side are numerous intercellular spaces occupied by bacteria. The bundle has been hollowed into a cavity, and at x, and y parenchyma cells are also occupied by the bacteria. These can be followed through a whole series of sections, but the method of entrance into these cells has not been made out clearly. Fixed in Carnoy 48 hours. Drawn with a Zeiss 16 mm. apochromatic and No. 12 compensating ocular. Slide 354-2, middle section, middle row.

The organism occurs singly, in pairs, and more rarely in fours joined end to end. Long chains and filaments have not been observed. Pseudozoogloæ or flocculent particles do not occur quickly in bouillon, but compacted masses have been observed on plates, etc., and they occur in the plant. Usually in fluid cultures no rim or pellicle is formed, but there may be flocculence in some liquids, e. g., Uschinsky's solution, though this is often wanting. Spores are not known to occur. This organism is a white, wet-shining, schizomycete, motile by means of 4 to 8 peritrichiate flagella (fig. 84). In young cultures motility is easy to observe. It has also been observed in slime taken directly from the vessels of the cucumber, melon, etc., and diluted with sterile water. In the plant motility is easier to observe in rods taken from tissues recently invaded than in those taken from crowded vessels. Involution forms occur in the plant and in various media—beet-juice, cucumber-juice, peptone-water, potato broth (vol. I, fig. 21), etc.

On steamed potato the growth is white and so closely like the substratum in color that it is scarcely to be distinguished therefrom except by its smooth, moist, glistening appearance. It has very little action on potato-starch, even very old potato-cultures reacting strongly with iodine. It does not soften the middle lamella of potato-cells. Potato-cylinders may or may not be grayed. In one experiment the organism remained alive on potato over seven months ( $16^{\circ}$  to  $18^{\circ}$  C.), but usually it is dead much sooner.

In one set of experiments no growth was obtained on red turnip-rooted table-beets, nor on turnip-roots, radish-roots, or cauliflower-petioles. When these experiments were repeated some years later the following results were obtained: On the beets growth was delayed but finally appeared. It



Fig. 83.†

was visible in one tube on the sixth day, in another on the fifteenth day, and in a third on the twenty-second day; the fourth tube remained sterile. Similar results were obtained by a repetition of the experiment: There was marked retardation of growth as compared with that in the agar-stab, none being visible on the beets the fourth day; on the thirteenth day there was a visible growth in one of the tubes but not in the other, on the twentieth day a typical growth appeared in the second tube. Upon radishes similar results were obtained, viz., marked retardation but final growth in most of the tubes. No growth was obtained on turnips (two sets) and only a doubtful growth on cauliflowers.

In 1896, inoculations were made into the juice of red table-beets filtered sterile and used both with and without the addition of calcium

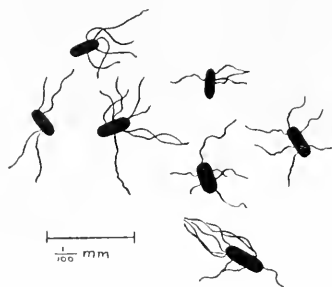


Fig. 84.‡

\*Fig. 82.—Bacteria from interior of cucumber-stem at time of general wilt of foliage, but while main axis was still green and normal in appearance (plate 1, fig. 2); bacteria were present in vessels in enormous quantities. The great mass of them were of size and shape of 2, those of size 1 being seen only occasionally. Drawn unstained with Abbe camera, Zeiss 3 mm. 1.40 n. a. apochromatic objective, and No. 18 compensating ocular. Anacostia, D. C., July 15, 1893.

†Fig. 83.—Free-hand drawings of paired rods of *B. tracheiphilus* which have lost their flagella. Stained by van Ermengem's nitrate of silver method in April, 1895, and measured after lying in balsam until Oct., 1895. Size of organism appears greater when stained in this manner than when stained without flagella-mordants. Very careful measurements of one member of each pair gave following result in microns:  $2.03 \times 1.05$ ;  $2.10 \times 0.98$ ;  $2.55 \times 1.10$ ;  $2.18 \times 1.05$ ;  $2.75 \times 1.20$ ; average  $2.32 \times 1.08$ . Circa  $\times 1500$ .

‡Fig. 84.—Camera drawing from cover-glass preparation of young culture of *B. tracheiphilus* stained by van Ermengem's nitrate of silver method. Flagella distinct; hundreds on cover-glass. Some rods with only one or two left, others with as many as 8; most bear about 6. An occasional flagellum is  $10.5 \mu$  long; most are shorter. Oct. 4, 1895. Circa  $\times 1500$ .

carbonate; also into boiled sterile beet-juice and the same rendered moderately alkaline by various small amounts of sodium carbonate, but in all cases growth was absent, feeble or long delayed. Involution forms were present.

The result of this series of experiments and of those which preceded it goes to show that the red beet either lacks some nutrient element or contains some substance which, while not destroying the germ, almost completely inhibits growth, and this whether the juice is acid or alkaline or whether sterilized by steam heat or by filtration. The result in tube 5, where after 25 days there was a considerable multiplication, seems to show that this inhibition is due to the presence rather than the absence of some substance.

The organism is white in the plant and also on a variety of culture-media. It produces no pigment other than the occasional gray stain on potato common to many bacteria.

In agar and gelatin the best growth along the line of the stab is at the top; the surface growth above the stab is thin, gray-white, and may eventually cover two-thirds of the surface (fig. 85*b*) or even the whole surface. Fig. 86 shows the appearance of a streak-culture on gelatin. The surface colonies on agar (figs. 87 and 88) and on gelatin are small, circular, slow of growth, gray-white, smooth and usually wet-shining. Internal striae may often be seen by careful manipulation of *reflected* light (fig. 89). They are not on the surface and can not be seen by transmitted light (fig. 90). Plate-cultures incubated at 25° C. are seldom in good condition for study before the sixth day. Often streak-cultures show a discrete growth either throughout or on the margins (fig. 91). It does not grow on strongly acid gelatin, or on the same after it has been made neutral or feebly alkaline to litmus but is still decidedly acid to phenolphthalein.

This organism is facultative anaerobic. It will not grow in the closed end of fermentation-tubes in sugar-free, peptonized beef-broth, nor in the same fluid with addition of milk-sugar, maltose dextrine (fig. 92*b*), glycerin (Vol. I, fig. 48), etc. When, however, grape-sugar, cane-sugar, fruit-sugar or mannit (?) are added to the beef-broth, the inoculated fluid becomes clouded in the closed end of the tube in the absence of air (see fig. 92*a* and Vol. I, fig. 47): No gas is formed in fermentation-tubes or in any of the common media, but inoculated culture-media containing grape-sugar, fruit-sugar, or cane-sugar become acid. This acid is not volatile, but rather is concentrated by boiling, and the writer found that it could be extracted from cultures in bouillon by prolonged shaking with ether.

In 1909, flask cultures several months old,† set in 700 cc. filtered river water containing 35 grams grape-sugar, 14 grams Witte's peptone, and 35 grams calcium carbonate, were submitted to Dr. Carl L. Alsberg for analysis. He did not succeed in identifying the acid, owing to insufficient amount of material, but made the following interesting negative determinations:

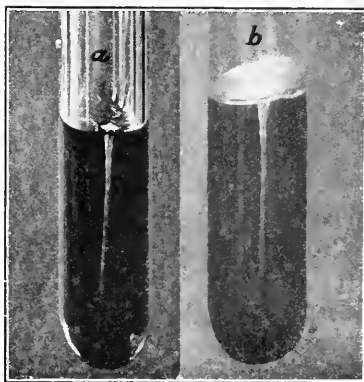


Fig. 85.\*

\*Fig. 85.—Gelatin stab cultures of *Bacillus tracheiphilus* after 10 days at 25° C.: *a*, inoculated June 14; *b*, June 18, 1904. No liquefaction. Figure *a* photographed under water, *b* photographed in air.

†The fluid was well clouded after some days and remained so for several months (more than 4). No pellicle formed, only small floating islands which were most abundant near the walls of the flask. There was considerable flocculent precipitate and the fluid became browner than that in an uninoculated flask. The organism at end of 4 months was still pure, living and virulent in flask A, as shown by cultures on agar and on potato, and by inoculations therefrom into cucumber. Eight plants were inoculated by needle-pricks in leaf-blades Nov. 29, and all contracted the disease within 10 days, while 35 check plants remained sound. The flasks B and C presented the same appearance as A, but were not tested until the end of 7 months and 14 months respectively, when the cultures were dead.

Sufficient acid was produced in 500 cc. [700 cc.] of the fluid to carry into solution 2.5 grams of CaO. This acid is not formic, acetic, propionic, butyric, or any other volatile fermentation acid. Neither did lactic, succinic, or oxalic acid occur in any appreciable amount. No amino acids were found. No alcohol and no volatile aldehydes were present.

Glycerin is fermented in presence of Witte's peptone with production of an acid, but this does not occur in the absence of air. Maltose-bouillon became feebly clouded in the closed end of fermentation-tubes after a week, but growth was so feeble that it was ascribed to impurities in the sugar. Similar results were obtained with mannit. In 1906 the experiments with maltose in fermentation-tubes were repeated using the melon-strain of the bacillus in peptone water with a maltose 3 times recrystallized in the Bureau of Chemistry. The result was clouding in the open end and **U**, but none in the closed end. In beef-bouillon and on steamed potato, in an atmosphere of hydrogen or of carbon dioxide, the organism makes some development but grows less well than in the air (fig. 93). Confinatory results were obtained with buried agar-streak-cultures (fig. 94).

The growth on agar is thin, white, wet-shining, with a distinct margin. The colonies do not grow rapidly. The best growth is usually at the bottom of the streak and in the upper part of the stab. Gelatin (figs. 85, 86), coagulated egg-yolk and egg-albumen, and Loeffler's solidified blood-serum are not liquefied. The growth on Loeffler's blood-serum was thin and poor. There was more growth in Dunham's solution made with Savory and Moore's brown peptone, which contains some muscle-sugar, than in that made with Witte's peptone. In Dunham's solution there was less growth than in corresponding tubes of *Bacillus amylovorus*. An organism identified as *Bacillus cloacae* grew well in Dunham's solution after *B. tracheiphilus* had ceased to grow in it. No indol reaction was observed (3 days) nor was any obtained from Dunham's solution after 15 days' growth.

In McConkey's bile-salt agar there was a moderate amount of smooth, wet-shining, surface growth, and some growth in the top of the stab, but no change in the color of the neutral red (20 days).

The organism is unable to grow in asparagin-water;† asparagin-water with dextrose; or asparagin-water with dextrose and nutrient mineral salts (asparagin being the only nitrogen compound). The growth in peptone-water (Witte's) with asparagin was not sensibly greater for some weeks than in simple peptone-water, but finally became greater in one tube, which afterward yielded a pure culture of *B. tracheiphilus*. This would seem to show that under some circumstances it may get its carbon food from asparagin, but not its nitrogen food. The experiment should be repeated. The organism refused to grow in filtered, boiled river-water containing 1 per cent sodium

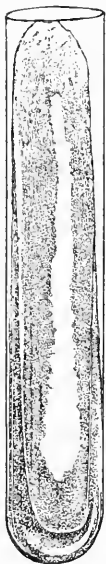


Fig. 86.\*

asparaginate, 1 per cent dextrose and 2 per cent glycerin. Neither would it grow in the same medium when ammonium tartrate or ammonium lactate was substituted for the sodium asparaginate. In another experiment, using distilled water, sodium asparaginate, dextrose and glycerin, and inoculating from potato very copiously, it clouded the fluid in the open end of the fermentation-tube slightly after a week, but never made a good growth. *B. tracheiphilus* will not grow in Cohn's solution, nor in acid (+33) peptonized beef-bouillon (acid of the beef-muscle). It grows in Fermi's solution and in Uscilinsky's solution, but rather feebly and with more or less flocculence. Potassium nitrate in peptonized beef-bouillon is not reduced to nitrite.

The growth of the organism in peptonized beef-bouillon is sensibly retarded by 1 per cent c. p. sodium chloride, and very decidedly by 1.5 per cent or 1.7 per cent. In most

\*FIG. 86. Streak culture of *B. tracheiphilus* on gelatin. Drawn from a photograph. Age about 14 days. No liquefaction.

†Repeated in 1906 with same negative result (20 days at 23° C., approximately).

instances 2 per cent sodium chloride in peptonized bouillon inhibited growth, but in one instance one tube out of five clouded thinly at the end of 12 days. The alkalinity of the bouillon used for inoculation seemed to play some part, *i. e.*, when inoculated from acid bouillon (+20) even 1 per cent sodium chloride inhibited growth (cucumber strain?). The organism is also sensitive to acids (fig. 95).

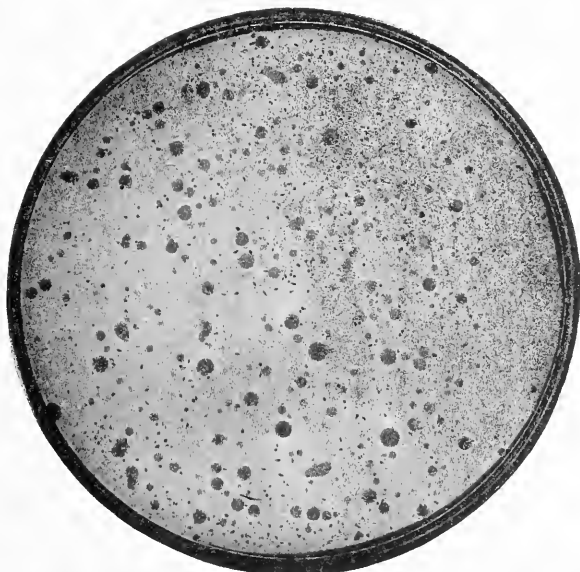


Fig. 87.\*



Fig. 88.†

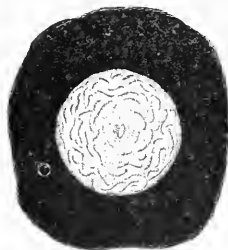


Fig. 89.‡

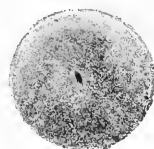


Fig. 90.§

\*Fig. 87.—Petri-dish poured plate of *B. tracheiphilus* after incubating 6 days at 22° to 25° C. On Jan. 27, 1904 with the usual precautions against surface contaminations, a tube of bouillon was inoculated with a little white, sticky slime from the interior of the inoculated cucumber-plant No. 552. Gradually the bouillon clouded thinly after the manner of a pure culture and on Feb. 2, plates were poured demonstrating its purity. The largest colonies were on the surface of the agar; the very small ones, in body of agar; and the thin ones, on lower surface of agar next the glass. Plates made from this same tube of bouillon on Jan. 27, *i. e.*, soon after introducing the viscid slime, remained sterile.

†Fig. 88.—Portion of an agar-plate of *B. tracheiphilus* showing appearance of buried colonies, surface colonies, and colonies breaking through to surface, after being incubated for 7 days at 30° C. Drawn Nov. 18, 1904, from plate 1 Nov. 11. ×2. Descended from rods which withstood freezing.

‡Fig. 89.—Colony of *Bacillus tracheiphilus* drawn by reflected light and magnified to show markings in the colony, its surface being smooth. This appearance is not visible by transmitted light, nor by looking across colony at an acute angle. Actual size of colony indicated by small circle. Plate I, Nov. 11, 1904, made from frozen bouillon and incubated for 7 days at 30° C.

§Fig. 90.—Surface growth of *Bacillus tracheiphilus* as seen under a low power by transmitted light, after 7 days at 30° C. Colony from same agar poured-plate as fig. 89. Actual size of colony shown by small circle. Drawn Nov. 18, 1904.

The optimum reaction for growth in peptonized beef-bouillon is about +8 of Fuller's scale. Growth on the acid side (acid of beef-juice) takes place up to about +28 (?); on the alkaline side (sodium hydrate) growth ceases at about -4 (?). These statements are to be taken only as general indications, for here again much depends on the original reaction of the culture-fluid used for the inoculation. Growth may be pushed farthest on the acid side by inoculating from acid bouillons, and on the alkaline side by inoculating from alkaline bouillons, e. g. -2 bouillon will cloud when inoculated from +2 or 0 bouillon, but not when

inoculated from +25 or +20 bouillon. The strain used for these experiments was the one which did not infect squash (1905).

In milk, growth continues for quite a long time but with no precipitation of casein or change in the appearance of the fluid. In litmus-milk there is little or no change of color, i. e., no decided reddening or bluing of the fluid, or loss of color (reduction) not even after several months. It has seemed to me at times that I could distinguish a slight change in the color of the litmus-milk (bluing), but if any it is so slight as to be readily overlooked. Milk is, therefore, a good differential culture-medium. In old litmus-milk-cultures (dried out two-thirds), on the walls of the tubes above the fluid, very small branching fern-like crystals (fig. 96) occurred after wetting the walls with the fluid, and these crystals did not appear in the 3 check tubes. On litmus-lactose-agar there is no change at first. After some time there may be a gradual deepening of the blue color, but never any reddening. This experiment was repeated in 1906 with the same result.

Only one bacillus more sensitive to dry air is yet known, viz., *B. carotovorus* Jones. In the writers' experiments, portions of solid cultures or fluid cultures were spread in thin layers and dried at room-temperatures on clean sterile cover-glasses and then tested by dropping from time to time into tubes of a bouillon known to be well adapted to the growth of the organism. In all cases the organism was found to be dead one-half hour to one hour after drying out, and in some

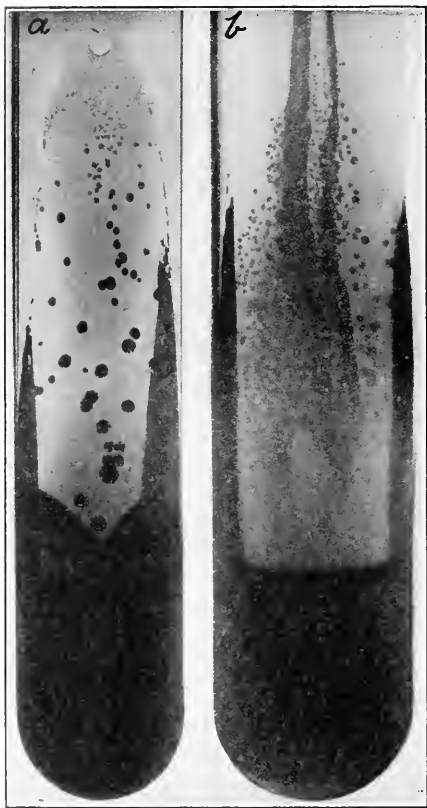


Fig. 91.\*

instances when taken from bouillon drying for so short a time as 15 minutes sufficed to kill it. Covers inoculated from the same bouillon and dried only for 10 minutes yielded cultures of the organism when thrown into bouillon. Much seemed to depend on the thinness of the layer. Possibly also the surface on which the bacillus is dried may exert an influence. The bacillus is also sensitive to sunlight (fig. 97.)

\*FIG. 91. —a, streak culture of *Bacillus tracheophilus* on litmus-lactose agar after 7 days, showing frequent tendency of organism to grow in discrete colonies. Tube 15, June 30, 1904. Photographed July 7.  $\times 1\frac{3}{4}$ . b, Same 8 days old at 22° to 26° C. In this tube it is also growing in the form of a streak. Tube 4, June 16, 1903. Photographed, June 22.  $\times 2$ .



The organism produces little or no odor. I have never been able to detect any, but the striped cucumber beetle seems to be able to do so.

Successful transfers of this organism were made from cultures exposed for twenty minutes to a very low temperature using a mixture of sulphuric ether and frozen carbon dioxide ( $-77^{\circ}\text{C}.$ ). At another time several experiments with liquid air ( $-119^{\circ}\text{C}.$ ) gave the same results, but quantitative tests showed the majority to have been killed. When exposed in bouillon over night to the temperature of liquid air, poured plates showed about 65 per cent of the organisms to have been destroyed (Vol. I, figs. 68 and 69). Tests a half year later, exposing in liquid air for half an hour, showed over 50 per cent to have been destroyed.

The minimum temperature for growth is (?)  $8^{\circ}\text{C}.$  In peptone-water, inoculated and kept in the ice-box at  $6^{\circ}$  to  $10^{\circ}\text{C}.$ , there was no clouding for 16 days, but on removal to room-temperature ( $25^{\circ}$  to  $26^{\circ}\text{C}.$ ) the tube clouded thinly in 48 hours and was subsequently used successfully for the infection of plants (page 271). At another time there was no clouding in 30 days at  $11^{\circ}\text{C}.$  to  $13^{\circ}\text{C}.$

The optimum temperature is, roughly,  $25^{\circ}$  to  $30^{\circ}\text{C}.$

The maximum temperature is  $34$  to  $35^{\circ}\text{C}.$  (?) *i. e.*, not determined accurately but somewhere around this point. In October, 1905, eleven tubes of  $+15$  peptone bouillon (stock 1622) were inoculated with the cucumber strain (from acid bouillon?) and exposed in the thermostat for 5 days at  $33^{\circ}\text{C}.$  and lower, gradually rising to  $36^{\circ}\text{C}.$ , but most of the time under  $35^{\circ}\text{C}.$  (The thermostat was stable but the night temperatures were not recorded, only assumed to have been like the day temperatures.) All of the tubes remained clear during the exposure and none of them clouded when removed to room temperature ( $25^{\circ}\text{C}.$ ). Two checks held at  $25^{\circ}\text{C}.$  clouded in 48 hours. This experiment was repeated three days later, paying careful attention to the night temperatures. Twelve tubes of bouillon were inoculated and exposed in the same thermostat for  $44\frac{1}{2}$  hours, after which they were removed and placed at  $25^{\circ}\text{C}.$  Two tubes were held as checks. The latter clouded in 48 hours. The heated tubes never clouded. The recorded thermostat temperatures were as follows:

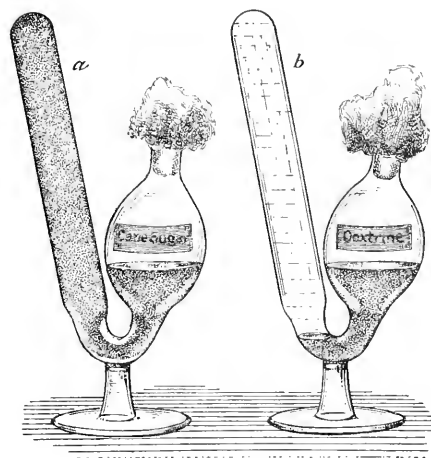


Fig. 92.\*

Oct. 14, 4 p.m. (after opening)	$34\ 10^{\circ}\text{C}.$
6 p.m.	$34\ 50^{\circ}\text{C}.$
9 p.m.	$34\ 50^{\circ}\text{C}.$
12 p.m.	$34\ 50^{\circ}\text{C}.$
Oct. 15, 4 a.m.	$34\ 50^{\circ}\text{C}.$
6 a.m.	$34\ 50^{\circ}\text{C}.$
Noon	$34\ 60^{\circ}\text{C}.$

Oct. 15, 5 p.m.	$35\ 50^{\circ}\text{C}.$
9 p.m.	$35\ 50^{\circ}\text{C}.$
Midnight	$35\ 50^{\circ}\text{C}.$
Oct. 16, 4 a.m.	$36^{\circ}\text{C}.$
6 a.m.	$36^{\circ}\text{C}.$
11 a.m.	$35\ 50^{\circ}\text{C}.$
12:30 p.m.	$35\ 80^{\circ}\text{C}.$

\*Fig. 92.—Growth of *B. tracheiphilus* in fermentation tubes: *a*, facultative anaerobically in cane-sugar bouillon, and, *b*, aerobically in meat-infusion with 1 per cent dextrose. The dextrose used was readily soluble in water and did not give a red reaction with iodine. Tube containing cane-sugar was inoculated Feb. 3, 1896, very copiously from tube 4, Jan. 21, a slant tube of sugar-agar inoculated from plant No. 246. The tube was doubtfully clouded in the open end on Feb. 6, and plainly so on Feb. 7. On Feb. 8 it was thinly clouded in the whole of closed end but the fluid was still alkaline. On Feb. 13 clouding was uniform in open end and closed end (nearly so on Feb. 10) and fluid was acid to litmus. On this date a transfer to potato yielded a pure culture of *B. tracheiphilus*. On Feb. 27 culture was dead, having been destroyed by its own acid. The dextrose-bouillon was inoculated May 8, 1895, and was clouded in open end May 13 but clear in closed end. On May 18 it was still clear in closed end and fluid was alkaline to litmus.

The thermal death-point is  $43^{\circ}\text{C}$ ., the lowest yet recorded for any organism infesting plants, and until recently the lowest known.

In an experiment made in June, 1896, an exposure of one hour to  $41^{\circ}\text{C}$ . killed all, *i. e.*, no colonies developed on the agar plate poured from the heated bouillon (11 days incubation) whereas the check-plate, *i. e.*, the plate inoculated from the tube before heating yielded several thousand colonies per square centimeter.

Exposure in bouillon for one hour at  $40^{\circ}\text{C}$ . killed three-fourths or more, as determined by agar poured-plates. Exposure to  $40^{\circ}\text{C}$ . for one-half hour destroyed about half.

In many ways *B. tracheiphilus* is a very sensitive organism, and consequently it is difficult to work with. It is hard to plate out owing to its viscidly. It is very sensitive to heat, to dry air, and to direct sunlight. Freezings are also harmful. It is sensitive also to its own decomposition products, especially acids. It is not a rapid grower nor a very copious one on culture-media and vigorous organisms crowd it out. It does not, however, lose virulence readily by cultivation on artificial media.

On most media, transfers must be reasonably frequent to keep it alive. It was alive once on sweet potato after 33 days. It was dead on carrot after 33 days. In one instance on steamed Irish potato, parts of a culture were alive after 26 days. In another instance a potato culture (which did not gray) was dead at the end of 16 days (see also plant inoculations, pages 275, 283). It has lived, however, in some of the writer's slant agar-cultures for several months and in litmus milk for 3 months; it may be kept alive for several months in peptonized beef-bouillon with addition of cane-sugar, if calcium carbonate is also added so as to neutralize the acid as fast as it is formed. Often from agar-stab-cultures a few months old it is recoverable, if at all, only by pouring sterile bouillon into the tubes and incubating for a week or more at  $25^{\circ}\text{C}$ . It is often recoverable from the interior of the plant only by direct streaks on potato or other suitable medium, or by putting the viscid slime into bouillon for 6 to 24 hours before attempting plates. In this case great care must be used to avoid surface intruders and the second or third dilutions, if they cloud, are best for the plates. In experiments made in May-June, 1895, the organism was dead in the upper part of agar-stabs at the end of six weeks at room temperature. The organism was alive in saccharose-peptone-bouillon after 10 days, but not after 24 days (acid was detected prior to the tenth day).

#### RÉSUMÉ OF SALIENT CHARACTERS.

##### POSITIVE.

A bacillus in the vascular bundles of cucurbits causing a wilt-disease; short rods (single, paired, in fours end to end, or in small clumps); motile, peritrichate; capsules; pseudozoogloecae; involution-forms; stains readily; smooth; white; viscid; glistening; slow grower on media; surface colonies small, round, discrete; no growth at  $37^{\circ}\text{C}$ . or at  $6^{\circ}\text{C}$ . (16 days); aerobic; facultative anaerobic (with grape-sugar, cane-sugar or fruit-sugar); from these sugars a non-volatile acid, soluble in ether; grows only in open end of F-tube with dextrine or glycerin, acid from glycerine; slime on steamed

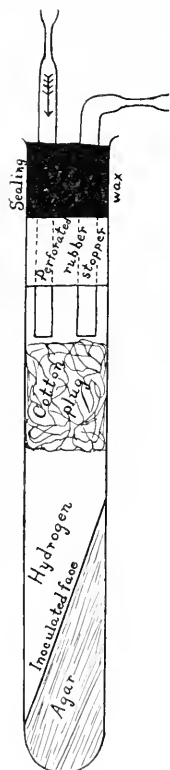


Fig. 93.\*

\*Fig. 93. —Method of making a culture of *Bacillus tracheiphilus* on slant agar in hydrogen. After thoroughly displacing the air, tubes were sealed in an open flame at the constricted parts. These tubes were about 9 inches long and 1 inch or more in diameter. In later experiments Novy jars were used.

potato is same color as the ungrayed substratum; usually it grays potato after a time; clouds peptone-bouillon and Dunham's solution thinly; growth retarded in acid juice of cucumber-fruits; also retarded or inhibited by juice of many other vegetables, *e. g.*, table beet, sugar-beet, turnip, etc.; grows on many media at 25° C., carrot, coconut, Fermi, Ushinsky, etc.; asparagin as carbon food (?); thermal death-point 43° C.; optimum for growth 25° to 30° C.; maximum, 34° to 35° C. (?); minimum (?) 8° or below; easily killed by dry air, sunlight, or freezing (50 per cent or more); ammonia-production (moderate); feeble production of hydrogen sulphide; in litmus-milk persistent growth without reduction or distinct change in color of litmus; short lived on many media; killed readily by acids, but lives long in cane-sugar-bouillon with carbonate of lime; grows on some media in hydrogen and carbon dioxide; dissolves middle lamella (cucumber-parenchyma); distributed by insects especially by *Diabrotica vittata*. Group No. 222.2322023.

#### NEGATIVE.

Mealy or dendritic surface growths; Gram's stain; endospores; chains; filaments; growth not yellowish, piled up or wrinkled; pellicle on bouillon; liquefaction (gelatin,\* blood-serum, egg-albumen, etc.); lactose and pure maltose in closed end of fermentation-tube; lab ferment; acid (in milk); gas (all media); pigment (except gray stain on potato); indol (?); nitrite from nitrates; starch-splitting; cellulose-dissolving (except possibly in host); asparagin as nitrogen food; ammonium salts as nitrogen food; steamed turnip, and cauliflower; Cohn's solution; acid bouillon (+33); acid gelatin; nearly odorless; not a soft rot; not infectious to tomato, potato, etc. On steamed potato liable to be confounded with a non-infectious coccus (follower) which reddens litmus milk.

Any organism which reddens or blues litmus-milk decidedly, reduces the litmus, throws down the casein, or clears litmus-free milk without precipitation may be set down at once as something else.

#### TREATMENT.

So far as known this disease is disseminated only by means of insects, consequently the first effort of the grower should be to reduce the number of these pests to the lowest possible terms. Various suggestions for the destruction of these insects have been made by entomologists, the most hopeful of which is perhaps, to trap the leaf-eating beetles by sowing between the rows or in the vicinity of the cultivated plants, other plants which these beetles are very fond of feeding upon. Subsequently both plants and beetles should

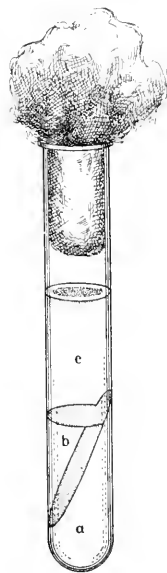


Fig. 94.†

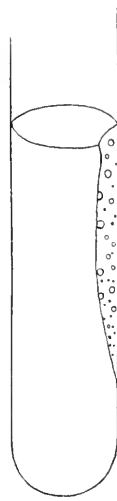


Fig. 95.‡

\*According to Barlow this organism grows better on potash gelatin than on ordinary gelatin, and slowly liquefies that medium both in plates and stab-cultures.

†Fig. 94.—Culture of *B. tracheiphilus* showing limited growth in the absence of air. A tube of nutrient agar was boiled one hour, slanted, cooled, and immediately streaked, forming *a*. Upon this *b* was then poured after boiling one hour and cooling to a safe temperature. As soon as *b* had solidified a third tube of agar was melted, cooled, and poured into the tube, forming *c*. In 4 days there was a distinct whitish growth the whole length of the slant. Subsequently colonies developed all through *b* and *c* but were visible to naked eye only at junction of *b* and *c* and in the upper 4 to 5 mm. of *c*, *i. e.*, in that part freest to absorb air. The agar contained beef-broth, Witte's peptone, and a moderate amount of sodium carbonate.

‡Fig. 95.—Stab-culture of *B. tracheiphilus* on acetic acid agar. Growth long delayed, discrete, and finally appearing mostly on one side where agar had shrunk from wall, *i. e.*, not in stab. This sketch shows appearance after 33 days.

be destroyed by sprays of kerosene or arsenates. Several successive crops of these "trap-plants" should be made to come on at short intervals. The first one should be planted some weeks in advance of the crop which it is desired to grow and the others at intervals of a few days. In this way most of these insects may be destroyed, especially if the growers of a whole neighborhood will combine. Squash-plants have been recommended as a trap-crop for the *Diabroticas*. There should be an abundance of these trap-plants, so that for some weeks they will offer a continuous feeding ground for the beetles. They are especially fond of collecting in the freshly opened flowers of the squash. Hand-picking at sunrise when these insects are sluggish and when they often congregate in large numbers under the leaves or inside of the flowers, should also be practiced systematically. Of course, in case of a disease of which insects are common carriers, much may be done to reduce its prevalence

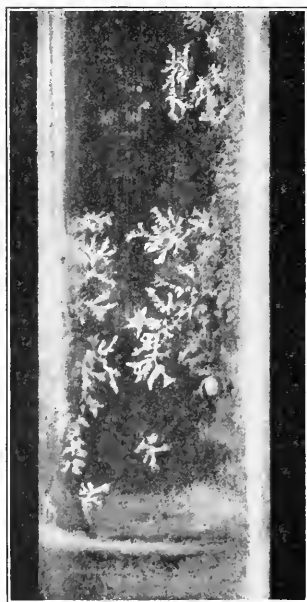


Fig. 96.\*

by systematically removing diseased plants as soon as the first signs appear, so that the opportunity for these insects to become contaminated, or for the bacilli to spread from these plants in any other way, is reduced to a minimum. Diseased plants should be pulled and burned at once or stored in some safe place and burned when dry. The disease is readily carried from one plant to another by insects, and this probably explains its appearance on fields not previously planted to cucurbits. If the disease has prevailed disastrously on any field the cultivation of other crops for some years is urgently advised. Cucumber-fruits are sometimes attacked and occupied by the bacteria, but it is not known whether the disease is transmitted to healthy fields through the agency of the seed-trade. The presumption is against this, because spores have not been found and because in some experiments it has been found that this organism is easily killed by dry air. It is too much, however, to assert absolutely that spores do not exist or that the disease can not be carried on seeds. Further studies are necessary. The organism does not grow at blood-temperature, and no harm is likely to ensue from the consumption of infected fruits.

The writer tried Bordeaux mixture without success, for this disease as it occurs in cucumbers near Washington, and Sturgis reported from Connecticut a similar want of success in melons. Additional trials are advised.

Experiments by the writer have demonstrated that occasionally the disease may be cut out by removing the inoculated leaves, soon after the first appearance of the wilt (p. 279). For field use, however, this method is not practical, owing to the fact that the bacillus advances down the vessels of the leaf at the rate of an inch or two a day and has usually entered the stem, before the farmer discovers and removes the wilting leaf. By the time the primary wilt has advanced so as to cover several square inches of the leaf-blade only a small proportion of the plants (cucumbers in my experiment) can be saved by removal of the affected leaves.

The disease is not to be feared in hothouses, if its appearance is recognized promptly, and if the insect carriers of infection are destroyed by the proper use of hydrocyanic acid gas. Otherwise an entire crop might be lost. Of course, diseased plants should be removed promptly and burned.

\*Fig. 96.—Three-months old culture of *B. tracheiphilus* in 10 cc. litmus milk showing delicate colorless crystals obtained by wetting sides of tube and allowing fluid to flow back. These crystals appeared in three cultures of this organism and not in three check tubes. Only one experiment, however. Photographed June 28, 1905. x2.

So far as known to the writer, no one has studied carefully the relative resistance of different varieties of melons, cucumbers, squashes, etc., to this disease. Possibly careful field work covering a number of seasons and many varieties would develop some interesting differences which might be turned to practical account in the production of resistant varieties by cross-breeding and selection.

To recapitulate.—*Prompt removal of diseased plants and wholesale destruction of cucurbitaceous insects are the best available means for holding this disease in check.*

#### PECUNIARY LOSSES.

This disease has proved an extremely vexatious one to a great many growers, but the writer has no means of knowing the full extent of the losses. Numerous complaints have reached the Department of Agriculture. The disease is particularly bad in some regions where cucumbers are cultivated extensively for pickles. The writer has seen entire fields



Fig. 97.\*

of cucumbers, of cantaloupes, and of winter-squashes destroyed by it in the vicinity of Washington, and knows from personal observations in other places (Delaware, New York, Michigan, etc.) that it is capable of doing serious damage over a large region of country.

According to Sturgis (1899) a destructive muskmelon disease in Connecticut is caused by *B. tracheiphilus*. "That this is actually the disease which, for the past five years at least, has destroyed a large percentage of the melon vines in Southern Connecticut there can be no doubt. Continuous observation in the field, in three separate localities, during the past season, convinced me that the chief source of trouble was the bacterial organism above mentioned."

\*Fig. 97.—Petri-dish poured-plate of nutrient gelatin densely sown with *Bacillus tracheiphilus*, covered with impervious paper except central star-shaped part which was cut away, and then exposed to sunlight for 3 hours at 15° C. There was prompt growth of the bacteria in the form of a white clouded mass in covered part, and almost no growth in part exposed to light, *i. e.*, only about 1 colony in 1,000 survived as shown by a count on the seventh day. The figure also illustrates a bit of bad technic. A moldspore germinated at *x*, and threatened to swamp the plate. When the cover was removed to cut it out the dish was exposed to a *draft of air*, the result being the entrance and growth of 30 or 40 other organisms so that when the plate was old enough to photograph (4th day) these also were visible.

In 1904 Paddock of Colorado, reported as follows: "The Hubbard squash is much subject to a bacterial blight, probably the same one studied by Dr. E. F. Smith. The growing of this crop is always precarious on this account. The disease was more or less abundant this season."

In 1905, Dr. B. M. Duggar informed me that the cucumber wilt disease, due to *B. tracheiphilus*, was extremely bad at Monroe City, Mo., and Palmyra, Mo., two nearby places, in 1903 and in 1904. He saw the disease there himself in 1904.

According to Kern (Indiana Plant Diseases, 1905, 1906) bacterial melon wilt prevails chiefly in the Central and Southern Counties of Indiana, often causing a total loss.

According to C. G. Woodbury, Associate Horticulturist, Purdue University Experiment Station (letter to the writer under date of November 16, 1909): "The bacterial wilt (*B. tracheiphilus*) causes an immense amount of damage every year to the cucumber and melon crop of this State."

In recent years many complaints have been received from Michigan, Illinois, Indiana, New York, Connecticut, etc. Probably it would be entirely safe to estimate the loss from this disease in the United States (where cantaloupes, cucumbers, pumpkins, and squashes are often cultivated on a very large scale) at not less than \$500,000 annually. At least one pickle-factory in the West was abandoned on account of its prevalence. The writer has not heard complaints from watermelon growers. The common wilt disease of that crop is due to a soil-fungus (see Bull. 17, Div. Veg. Phys. and Path., U. S. Dept. of Agric., 1899).

#### HISTORY.

So far as known there is nothing definite in scientific or horticultural literature respecting the occurrence of this disease prior to the beginning of the writer's experiments on it in 1893 and since that date it has received, so far as known to the writer, no serious attention from any other plant pathologist.

In 1908, Troop and Woodbury endeavored to get some further light on the transmission of this disease by soil or insects, but owing to defective screening (which permitted the entrance of *Diabrotica vittata*) the experiment miscarried, but is not wholly devoid of interest. Melon vines to the number of eighty were planted, two in a pot, in soil from a field where all of the plants had died of the bacterial wilt in 1907. The pots were set together in a field which had never borne plants subject to this disease. Half of them were screened, half exposed freely. The soil in half of the pots of each lot was steamed previous to planting (which would destroy any of the bacillus present in it). The wilt disease prevailed extensively in each one of the four groups of plants—most in the uncovered plants, and in those on the steamed soil. In each case the ratio was about 2 : 1. Altogether, including the replants attacked, there were 60 cases.

So far as any conclusion can be drawn from these experiments it is confirmatory of the writer's view first advanced in 1895 that this disease is distributed by *Diabrotica vittata*.

## LITERATURE.

1893. SMITH, ERWIN F. Two new and destructive diseases of cucurbits: 1. The muskmelon Alternaria; 2. A bacterial disease of cucumbers cantaloupes, and squashes. Paper read Aug. 21, 1903, before Sect. G. Am. Asso. Adv. Sci. at Madison, Wis. Proc. Am. Asso. Adv. Sci., 42nd meeting, for 1893. Salem, 1894, pp. 258-259. Also a separate. Brief abstract in Bot. Gazette, Sept., 1893, p. 339.
1894. HALSTED, BYRON D. Fungous diseases of the muskmelon. 14th Ann. Rept., New Jersey State Agric. Exp. Sta. and the 6th Ann. Rept. New Jersey Agric. College Exp. Sta. for 1893. Trenton, 1894. Part II, pp. 353-355. 1 fig. of leaf. See also p. 426 at bottom.
- Describes a disease of muskmelons said to be widely prevalent and attributes it to bacteria. From a statement on the bottom of p. 354 respecting the appearance of some of the leaves it is possible that a portion of these plants were attacked by *Bacillus tracheiphilus*, but all could not have been, since this organism does not cause a moist soft rot of the stems such as Dr. Halsted particularly describes. He says it is a disease complained of especially in the South (Mississippi) and also that "This disease is quick acting, and healthy tissues, when inoculated, soften and decay in a few hours." Possibly this disease was due to the soft rot organism since described by Ciddings as *Bacillus melonis*.
1895. SMITH, ERWIN F. *Bacillus tracheiphilus* sp. nov., die Ursache des Verwelken verschiedener Cucurbitaceen. Centrallbl. f. Bakt., 1895, 2 Abt., Bd. I, No. 9-10, pp. 364-373. Also a separate.
1896. SMITH, ERWIN F. The path of the water-current in cucumber plants. \* \* \* 5. The result of parasitic plugging of the vessels. *American Naturalist*, July 1896, pp. 561-562.
1897. SELBY, AUGUSTINE D. Certain troublesome diseases of tomatoes and cucurbits. Proc. Columbus Hort. Soc., Quarterly Jour. of Proc., Columbus, 1897, No. 4, Ann. Rept. for year ending Dec. 31, 1896 (vol. XI), p. 113. Also a separate.
- Reports the occurrence in Ohio of cucumber-wilt due to *B. tracheiphilus*; "The watermelon also suffers from attacks of *Bacillus tracheiphilus*, Smith" (p. 115).
1897. SELBY, A. D. Diseases of cucurbits, 1. Bacterial blight (*Bacillus tracheiphilus*). Bull. 73, Ohio Agric. Exp. Sta., Wooster, Ohio, Dec. 1896, pp. 233-235. Printed at Norwalk, Ohio, 1897.
- "The bacterial disease of muskmelons \* \* \* has proven destructive at most points in the State."
1897. SMITH, ERWIN F. The spread of plant diseases: A consideration of some of the ways in which parasitic organisms are disseminated. Lecture delivered before the Mass. Hort. Soc., March 27, 1897. Proc. of the Society for 1897, printed in 1898. Also a reprint from the same, pp. 9.
- An abstract of the lecture appeared in one of the Boston papers soon after its delivery, and there was also a separate abstract.
1898. SMITH, ERWIN F. Some bacterial diseases of truck crops. Trans. of the Peninsula Horticultural Society, Meeting at Snow Hill, Md., Jan. 11-12, 1898. Also a separate pp. 142-144.
1899. STURGIS, W. C. Some common diseases of melons. 22d Ann. Rept. Conn. Agric. Exp. Sta. for 1898, Hartford, 1899, pp. 225-228, 234, 235.
- The first three pages are on bacterial wilt. Spraying experiments in several localities were made with Bordeaux mixture, potassium sulphide, sulphur, and Laurel green, but no conclusive results were obtained. "It is probable that the susceptibility of melons to contract the bacterial wilt is unaffected by the fungicides commonly used against fungous diseases. Removing and destroying all wilted vines is the only practical method of preventing the spread of the disease."
1899. SELBY, A. D. Further studies of Cucumber, Melon and Tomato diseases. Ohio Agric. Exp. Sta. Bull. 105, Columbus, Ohio, 1899, 8 vo., p. 221. [A brief note.]
1899. IWANOFF, K. S. Über die Kartoffelbakteriosis in der Umgegend St. Petersburgs im Jahre 1898. Zeitschrift f. Pflanzenkrankheiten, 1899, Bd. IX, p. 131.
- Author also reports having found *B. tracheiphilus* doing much damage on cucumbers in 1898 in Russia near St. Petersburg.
1900. STONE, GEORGE E. AND SMITH, R. E. The bacterial cucumber wilt. 12th Ann. Rept. Hatch Exp. Sta. of Mass. Agr. College, Jan. 1900, p. 57.
- Reports occurrence of *B. tracheiphilus* on cucumbers at Amherst, Mass., in 1899.
1901. GARMAN, H. Enemies of cucumbers and related plants. Kentucky Agricultural Experiment Station Bulletin No. 91, Lexington, Kentucky, March 8, 1901.
1901. SMITH, ERWIN F. Entgegnung auf Alfred Fisher's "Antwort," etc. Centrallbl. f. Bakt., 1901, 2te Abt., Bd VII, Nos. 3, 4, and 5-6. Also a separate.
- Plates 1 to vii, and the accompanying text, pp. 139 and 190 to 195, relate to *B. tracheiphilus*. With one exception the plates are heliotype from photomicrographs by the writer.
1904. CLINTON, G. P. Squash Wilt. *Bacillus tracheiphilus* Sm. Report of the Conn. Agric. Exp. Sta. for 1903. New Haven, 1904, Part IV, Report of the Station Botanist, p. 359.
- "Summer and Hubbard squash, also muskmelons and cucumbers, are subject to this wilt, which last year was more common than usual." An excellent figure of wilting and wilted squashes is given.
1907. KIRK, T. W. Bacterial Wilt of Cucumbers. Ann. Report, New Zealand Department of Agriculture, 1907, vol. XV, p. 158.
- Kirk believes that he has observed the wilt of cucumbers due to *Bacillus tracheiphilus*, in New Zealand, but the specific organism was not isolated.
1907. EDWARDS, S. F. *Bacillus tracheiphilus*. Thirty-second Annual Report, Ontario Agric. Col. and Exp. Farm, 1906. Toronto, 1907, p. 137.
- "It is difficult to isolate this bacillus or to keep it alive, for it grows feebly or not at all in the ordinary media of the laboratory, especially in gelatin media. We have devised, prepared, and tried many special media and have found several in which the organism grows freely, the growth in certain gelatin media being especially copious and characteristic."
1909. SACKETT, WALTER G. Wilt of the cucumber, cantaloupe and squash. Colorado Agric. Exp. Station, Bull. 138, Jan. 1909, pp. 22-23.
1909. TROOP, J. AND WOODBURY, C. G. Melon Wilt. Indiana Agric. Exp. Station, Twenty-first Annual Report (for 1908), Lafayette, 1909, pp. 30-31.
1910. SELBY, A. D. Brief handbook of the diseases of cultivated plants of Ohio. Ohio Agricultural Experiment Station Bulletin 214. Wooster, Ohio, March, 1910, p. 394.

## BLACK ROT OF CRUCIFEROUS PLANTS.

(*Synonyms:* Dry rot, Brown rot, Stem-rot, Black stem, Black vein.)

### DEFINITION.

This is a specific communicable disease of the cabbage and its allies, usually requiring, except in seedlings, several months for the crippling or entire destruction of the plants. When not accompanied by other bacteria, the signs are dwarfing or one-sided growth, yellowing, gradual loss of leaves, and a brown stain of the vascular system, which is the primary seat of the disease (plate 17). Closed bacterial cavities are common in the bundles. Frequently, secondary parenchyma-rots set in and then the destruction of the plant is more rapid, and its appearance is changed.

### HOST-PLANTS.

This disease has been observed by the writer in cabbages (*Brassica oleracea* f. *capitata*), cauliflower (*Brassica oleracea* f. *botrytis*), collards (*B. oleracea* f. *gemmifera*), kale (*Brassica oleracea* f. *acephala*), kohlrabi (*Brassica oleracea* f. *gongylodes*), rape (*Brassica napus*), turnip (*Brassica rapa*), rutabaga (*Brassica campestris*),\* charlock (*Brassica sinapistrum*), and radish (*Raphanus raphanistrum*). With exception of collards and charlock the writer has inoculated it successfully into all of the above plants, and repeatedly into cabbages. It is the same organism in all of these species and varieties. On some of these plants numerous infections have been obtained also by other persons. The writer has produced the disease also in the black mustard (*Brassica nigra*) by pure culture inoculations. Very likely other cruciferous plants will be found to be subject to this disease.

The following species were inoculated by the writer by needle-punctures in stems and leaves, but did not contract this disease: *Hyacinthus albus*, *Solanum tuberosum*, *Cucumis sativus*, *Matthiola annua*, *Nasturtium officinale*, and *Nasturtium armoracia*. These experiments should be repeated on *Matthiola* and on the *Nasturtiums*, particularly on *N. armoracia*, because the latter plant is known to be subject to a disease of the crown and root which results in brown-stained vessels and cavities of considerable size (Sorauer) and because Faber, of Berlin, has recently reported its occurrence in stock (*Matthiola*). The writer has himself seen this disease of horse-radish in the United States, but not under conditions that made it possible to determine its cause.

### GEOGRAPHICAL DISTRIBUTION.

This disease has been observed in many parts of the United States. It occurs in Vermont, New York, New Jersey, Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, Alabama, Florida, Kentucky, Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, Iowa, Nebraska, Colorado, Texas, Arizona, Washington State, and California(?). In 1904 it was observed by the writer in cabbage, cauliflower, and kohlrabi at Miami in Southern Florida, and what seemed to be the same thing was found the same year in cabbages planted in gardens at Baracoa in the extreme eastern end of Cuba (latitude 20° 35'). It has been reported also from Porto Rico. The disease was first described from the United States, but it has since been shown to occur in various parts of Europe, e. g., in England, Germany, France, Switzerland, Denmark, Holland, Austria and Russia (Frank, Harding, Appel, Hecke, van Hall, Potter, Brenner, etc.). The writer searched in vain for it in southern Italy in 1906. The gardens of cauliflower about Naples showed no trace of it. Nothing is known as to its occurrence in other parts of the world, except that Kirk has recently reported it from New Zealand. The disease is to be looked for wherever plants of this family are cultivated.

\*The preceding 8 forms are closely related and are all classed under *B. campestris* L. by some authors.







## SIGNS OF THE DISEASE.

There is usually little difficulty in determining the existence of this disease. It is not a soft rot, although it may be complicated by the appearance of soft rots. A striking characteristic, especially in cabbages, is the yellowing of the foliage accompanied by a black stain in the vascular system. This stain in the veins often causes patches of the leaf to appear as a conspicuous black network on a yellowish or light brown background (fig. 98). The reader may consult also the colored figure in *Centralb. f. Bakt.*, 2 Abt., Bd. III, Taf. VI. Such leaves are not wet or decayed but have a rather dry, somewhat leathery appearance. When badly diseased, there is a gradual or successive shedding of such leaves, so that the cabbage plant or cauliflower plant (fig. 99) may come to have a small terminal tuft of leaves often more or less distorted, and separated from the root by a long stem bearing the conspicuous scars of many cast-off leaves. The stems of such plants are browned internally in the vascular ring (fig. 100 to 104). On cabbage-stems, etc., which have lost many leaves there is frequently a slight pushing of shoots from the axillary buds, but it is not known whether such growths are stimulated by the presence of the bacteria, or are due solely to an unusual loss of leaves, the latter being the most probable (fig. 105x). When the cabbage is attacked early in the season and severely, it is either destroyed outright in the course of a few weeks (seedling stage), or is so injured that no head forms. Dwarfing is a common sign of this disease. Very often the plant is attacked more on one side than on the other, the result being unequal growth and a small imperfect head.

The thick petioles of infected leaves may show no external evidence of the disease, but if examined in cross-section, the vascular bundles or leaf-traces will be found to be black or brown and occupied by bacteria (fig. 102). On slender petioles these blackened leaf-traces may show through as dusky stripes; these pass into the stem, which on cross-section is found to have a blackened or browned woody cylinder. This stain in the main axis may involve the entire circumference of the xylem-cylinder or be confined to one side or even to a few vessels on one side of the stem, the amount of stain depending on the place of infection, on the level at which the section is made and on the stage of progress of the disease, which is extremely slow in hard dry tissues and not very rapid even in soft watery ones. Usually the bark and pith of such stems are free from bacterial occupation and normal in appearance but not always (figs. 103, 105). After the first month or two the main roots

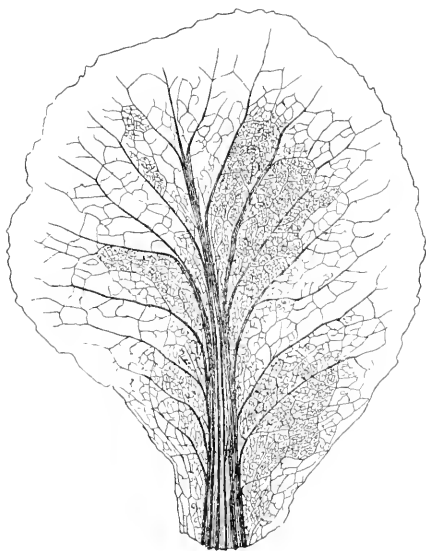


Fig. 98.\*

\*Fig. 98.—A cabbage-leaf with veins in middle part conspicuously blackened by *Bacterium campestre*. Marginal portion free from signs of disease except toward base. The result of a pure culture inoculation made upon another leaf of same plant by means of needle-punctures. The course of the organism was down the vascular system of the inoculated leaf into the stem, up vessels of the stem and out into the vascular system of this leaf. The freedom of the margin of the leaf from black venation is due therefore to the fact that the bacteria had not yet reached the final ramifications of the vascular system, at least not in numbers sufficient to brown the veins. July, 1897.

of cabbages and the basal part of the stem are generally quite woody; these parts, therefore, are not usually affected except in young plants. Sometimes a well-grown cabbage plant badly diseased in the foliage will show a few insignificant black specks in its woody base, but more commonly there will be none whatever unless the plant has been diseased from the time it was a seedling. In turnips the most striking signs are usually in the fleshy root which may not be well developed, if attacked early in the season, and which is often hollowed out into considerable cavities even when there are no external signs other than dwarfing and



Fig. 99.\*

leaf-injury (fig 106). In turnips examined by the writer in August, 1896, the disease had so seriously interfered with growth that the underground parts were more like carrots in shape than like turnips. In the cauliflower and other plants mentioned the signs are much the same as in the cabbage, the black veining being more or less conspicuous, as the case may be. Marginal leaf-infections are very common in cabbage (plate 2 and fig. 9) and in charlock. Thousands of such infections have been observed by the writer. In kohlrabi the black

\*FIG. 99.—Effect of bacterial black rot on cauliflower. Plants very badly dwarfed; many leaves fallen. Collected at Miami, Fla., Mar. 1904.

venation of the fleshy edible part is very conspicuous (fig. 104) although externally this portion of the plant may appear perfectly sound, the infection having taken place through the leaf-traces. Cavities also appear in the flesh of the kohlrabi (fig. 107). In those investigated by Hecke in Austria and those seen by the writer in Florida, the fleshy part was not dwarfed and was sound externally. In turnip roots a water-soaked appearance of the flesh is not infrequent, but the brown stain also occurs. On cross-section a yellowish bacterial slime frequently oozes from the blackened bundles of badly diseased stems. The writer observed this ooze very frequently in charlock. The unmixed disease has no conspicuous odor except possibly in turnips, but when the secondary white, rapidly disintegrating soft or wet-rots set in the smell is usually very disagreeable.

The only serious malady of cabbages likely to be confused with this is a *Fusarium* disease first described by the writer (Bull 17, Wilt disease of cotton, watermelon and cowpea, 1899, footnote, p. 41) and more recently by Mr. Harter (Science). This occurs from New

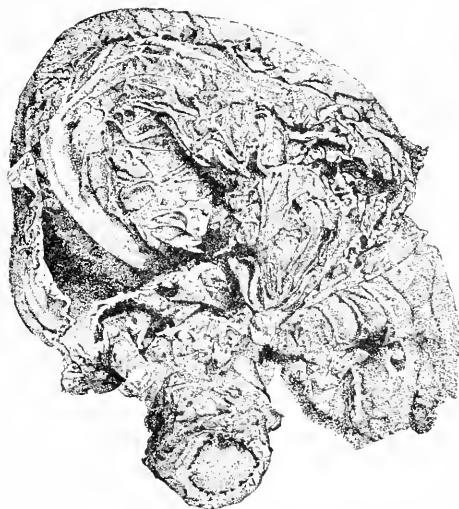


Fig. 100.\*

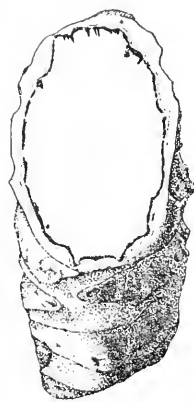


Fig. 101.†

York to South Carolina and is an almost equally serious disease, but is easily distinguished by the presence of the fungus in the vascular system. In the absence of a microscope, bad cases of the two diseases can usually be distinguished by cross-cutting the stems with a clean sharp knife and leaving them under a clean bowl or pan for 24 hours in a moist warm place. In the one case there is then often a slight yellowish ooze from the blackened bundles; in the other case there will be often a ring of white fungous threads extruded from the brown woody cylinder.

Slowly extending and relatively unimportant marginal leaf-injuries due to fungi and to other bacteria have been seen by the writer occasionally on the cabbage, and in some fields these might be mistaken perhaps for the marginal infections of the black rot, particularly by persons not very familiar with the latter. A little experience will generally enable

\*Fig. 100.—Cabbage head showing in the stem a conspicuous ring of black bundles due to *Bacterium campestre*. From a field in Holland. After van Hall.

†Fig. 101.—Cauliflower-stem parasitized by *Bacterium campestre*. Organism confined to vascular bundles, which are stained black. Beeville, Tex., Dec. 1902. Natural size.

one to discriminate. The lapse of a little time between inspections will also help one to judge, since in its further progress the black rot is quite different from the other foliar diseases. In this connection see plate 18.

### ETIOLOGY.

The cause of this disease is a yellow one-flagellate micro-organism, *Bacterium campestris* (Pammel) EFS. Conclusive proof of the infectious nature of this organism was obtained

on turnips by Pammel in 1893 and published in 1895. He performed 20 *direct* inoculations successfully. Subsequently he isolated the organism. His pure cultures were derived from turnips and rutabagas, and 8 successful inoculations were made into rutabagas, as many plants being held for control. The signs of the disease appeared in the course of a few days, finally involving the whole plant; this same organism was subsequently isolated from the diseased tissues, *i. e.*, from the blackened bundles and advancing margin of the rot.

In 1896-97 the writer repeated and confirmed Pammel's experiments on turnips and rutabagas and extended the inoculations to cabbages, cauliflower, kale, rape, radish, and black mustard. Two strains of the organism were used for most of these infections, *viz.*, pure culture isolations from diseased turnips obtained in Maryland and similar cultures from diseased cabbages from Wisconsin, but some cross-infections were also made with the organism obtained from charlock in Wisconsin. Microscopic examinations, bacteriological cultures and cross-inoculations showed the disease to be identical in all of these plants.\* He also confirmed and considerably extended Pammel's description of the organism. The writer at this time had obtained altogether more than 60 successful infections resulting in typical cases of the disease. From diseased plants at long distances from the point of inoculation he several times re-isolated the organism and obtained additional infections with such cultures. Only one of his many control plants contracted the disease and this under circumstances which pointed clearly to neighboring inoculated plants as the source of the infection and to mollusks (*Agriolimax agrestis*) as the carriers of the bacteria.

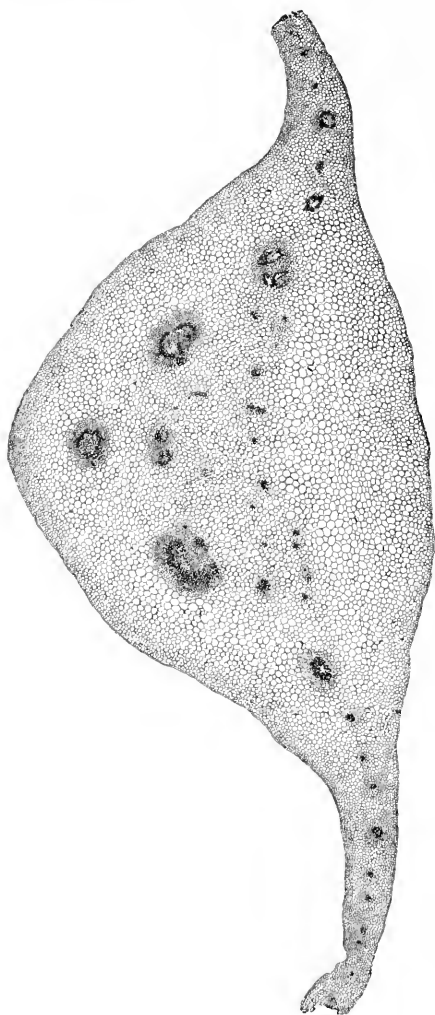


Fig. 102.†

\*Punctures with a sterile needle never induced any disease in my experiments. Hecke also mentions this fact particularly.

†FIG. 102. Cross section of petiole of cabbage, showing every bundle blackened by *Bact. campestris*, the parenchyma being free. Slide 8, plant No. 42.



Ragged leaf disease of cabbage associated with a slow bacterial decay of margins of leaves (not due to *Bact. campestris*). No heads were formed. Hothouse Jan. 18, 1906.





In Wisconsin, Russell & Harding repeated the experiments of Pammel, and of Smith, and confirmed their conclusions respecting the bacterial nature of the disease. Harding also studied the morphology and physiology of the organism quite carefully. The number of their successful infection experiments amounted to several hundred. In New York and in Europe, Harding subsequently continued his studies. On his return from Europe he obtained numerous successful infections on cabbage and cauliflower with a culture which he isolated from a diseased cabbage-plant found by him in Switzerland. Comparative tests were also instituted and neither in its cultural characters nor in its infectious properties was any difference detected between the Swiss organism and the one from New York or Wisconsin.

Hecke subsequently discovered the disease in kohlrabi in Austria and published two instructive papers on it, the number of his infection-experiments exceeding 100. Russell, however, was the first to obtain the disease in kohlrabi by inoculations (Bull. 65, p. 22). Van Hall then studied it in cabbages in Holland. More recently Brenner in Basel, under direction of Dr. Alfred Fischer, investigated the etiology of this disease, and after experimenting for two seasons states that he can only confirm Smith's conclusions respecting the cause of this disease.

The writer has himself isolated this organism from diseased plants obtained from Illinois, Wisconsin, Michigan, Ohio, Pennsylvania, Western New York, Long Island, Maryland, Alabama, Florida and Texas, and has studied the disease in the field in half a dozen different States. He has also produced the disease in cabbage by inoculating with a pure culture of the organism received from Hecke, who isolated it from kohlrabi grown in Southern Austria and himself proved its infectious nature on a variety of crucifers. In most of the above mentioned isolations by means of poured plates, *Bacterium campestre* was found in the vessels of the plants in pure culture. Only occasionally were mixtures obtained and even then the yellow organism was the preponderant form.

Plants are attacked by this disease in all stages of growth from seedlings in the seed-bed to plants ready for the market. In all this class of plants most of the infections, perhaps all of them in plants beyond the seedling-stage, are through the parts above ground and generally by way of the leaves. The writer, who has spent many weeks in cabbage-fields, has never seen anything in midsummer or later suggestive of infection through the root-system, *i. e.*, roots diseased and parts above ground not diseased, and Hecke's experiments of growing plants in soil mixed with tissues swarming with the organism, yielded only negative results. So did my own. Brenner also made similar experiments with similar results:

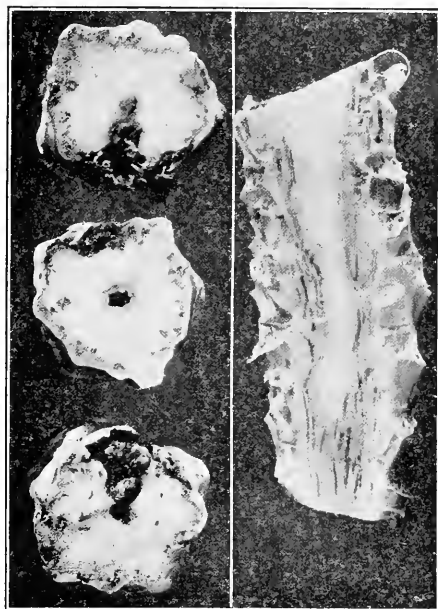


Fig. 103.\*

\*Fig. 103.—Longitudinal section and cross-sections of stems of cauliflower diseased by *Bacterium campestre*, showing stain and cavities in the stems. Miami, Fla., March, 1904.

The plants did not contract the disease even when the root-system was wounded. Russell also states that the disease does not find its way into the plant through the root-system. Stewart and Harding believe, however, that it may be communicated through the root-system, and this is not at all unlikely in early stages of growth when the tissues are soft. Later the woody stem offers an impassable barrier. Potter states that the disease has occurred repeatedly in Northumberland, England, in Swedish turnips, but that he observed it only on the root after it was well developed and always beginning in a local root-injury of some sort.

Infections above ground take place readily through wounded surfaces and the organism which causes the disease may be disseminated by a variety of leaf-eating insects (fig. 108),

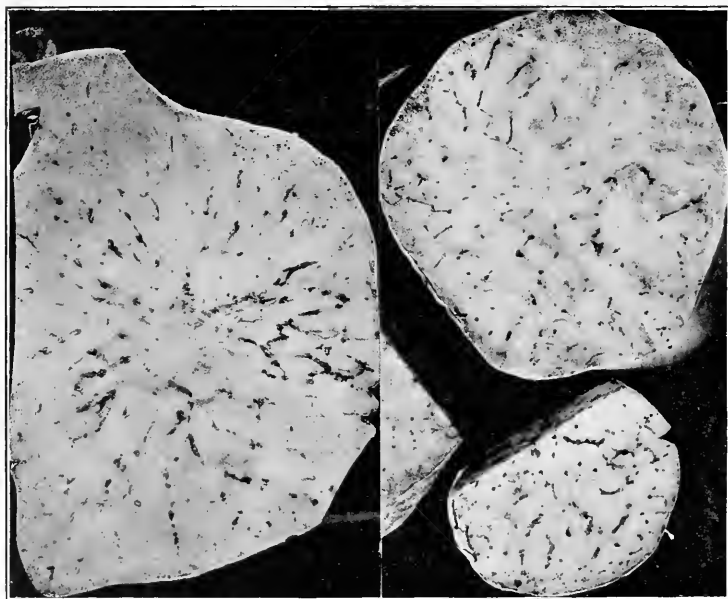


Fig. 104.\*

either by being introduced directly into wounds or more often perhaps by being left here and there on the uninjured margins of the leaves subsequently to find its way into the plant in the manner next to be described. Probably the leaves of plants are occasionally infected from the dust of the fields. The disease does not appear, however, to be one which is spread widely through the medium of the air. At least, as already recorded (Farmers' Bulletin, January, 1898) the writer has seen fields nearly free from the disease with only a fence separating them from fields in which half the plants were badly diseased and had been for many weeks, while multitudes of new infections were taking place right and left. This field also contained a multitude of infected weeds (charlock).

In hothouse experiments the writer succeeded in transmitting the disease by means of the larvæ of the cabbage butterfly (*Plusia*) and by slugs (*Agriolimax*). Brenner con-

\*FIG. 104. —Sections of kohlrabi, showing blackened vascular bundles due to *Bacterium campestre*. Photographed by the writer at Miami, Fla., March, 1904. The right and left were from different plants. See fig. 107.

firms infection by mollusks and reports successful transmissions by aphides. He recovered a yellow Schizomycete from the body of an aphid which had punctured a diseased spot, and with this organism he induced the disease on sound plants. A curculio (*Contrachelus*), which lays eggs in the stems of young cabbage plants, is also open to suspicion. Any creature which gnaws diseased leaves or stems and then gnaws or even crawls over healthy ones is very likely to transmit the infection. It is desirable that further studies should be made, especially on plants in the seed-bed and soon after transplanting, particularly with reference to underground infection.

Wounds are not necessary, however, for the transmission of this disease, nor do the majority of cases arise as a result of trauma.

The greater part of the infections (Smith, Russell, Hecke) unquestionably take place through certain natural openings of the plants, known as water-pores. These are modified stomata which occur in groups on the teeth of the leaf and through which excessive moisture taken up by the root-system is extruded from the plant. When the air is warm this moisture is given off as an invisible vapor, but during cool nights it gathers on the leaf-serratures in drops like dew, and may persist for hours after sunrise.

It was experiments with slugs which led the writer to the discovery of water-pore infections. On leaves which had been bitten and infected by *Agriolimax*, a few belated infections appeared on the leaf-margins where no wounds could be detected. A study of these infections showed that they began in the leaf serratures. This placed the question of natural infection in a wholly new light and led to further experiments with the results already known (vide *Centralt. Bakt.* 2 Abt., 1897, page 411). Up to the time of the preparation of that paper the writer had not studied this disease in the field and his conclusions were based only on experimental data. It was therefore observed with the greatest interest, in the summer of 1897, how well the field observations bore out the conclusions of the laboratory and hothouse.

In the last (second) edition of his "Vorlesungen" Fischer has questioned the possibility of general infection through the water-pores on two *a priori* grounds: (1) There is very little nutrient material in the fluid extruded from the water-pores; and (2) it would

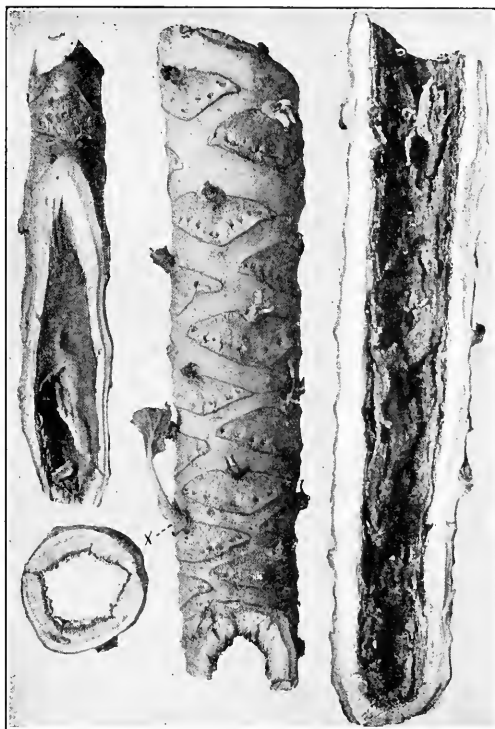


Fig. 105.\*

\*FIG. 105.—Stems of collards (*Brassica oleracea* f. *gemmifera*) from Tampa, Fla., badly affected by the black-rot. Pure cultures of *Bact. campestris* were plated from the interior of young shoot at point marked X (see fig. 128). Photographed Sept., 1902. Natural size.

require quite a good many hours for a dust-dry bacterium to become sufficiently moistened so as to multiply and enter the sub-stomatic chamber. A sufficient reply is that: (1) the organism does multiply considerably in this extruded fluid, as the writer demonstrated *in vitro* in 1897, "very little" nutrient material being *sufficient*, and (2) the hypothetical, dust-dry, wind-borne bacterium requiring a half day or more to moisten it, is probably not the one that usually enters the water-pores and induces the disease, but rather a fresh germ recently come from the interior of some affected leaf as an extrusion from some water-pore already diseased, or left in the vicinity of the water-pore by some wandering insect, which during its feedings on diseased leaves has first contaminated its own body and then various uninjured parts of the same plant and of other plants; such a bacterium would be ready to grow as soon as it found lodgment in a moist place. These mountains of difficulty therefore disappear as soon as the actual conditions are known.

Water-pore infections take place only when the weather conditions are such that the

extruded fluid from the plant remains over the water-pores for some hours in the form of drops. Moist weather with a day temperature of 20° C. appears to be very favorable for infection. Under these circumstances if any living rods of this organism happen to be lying in the vicinity, so as to be wetted, they are stimulated into growth and, being motile, they find their way readily into the sub-stomatic chamber. Proof of water-pore infections was furnished by the writer in August, 1897, and subsequently by Russell, by Hecke, and by Brenner.\* Hecke made water-pore inoculations on 14 kohlrabi plants, of which only 2 were entirely negative while 8 were very successful. The period of incubation, that is, the time from the entrance of the organism to the appearance of the disease in the veins of the cabbage-leaf, is usually several

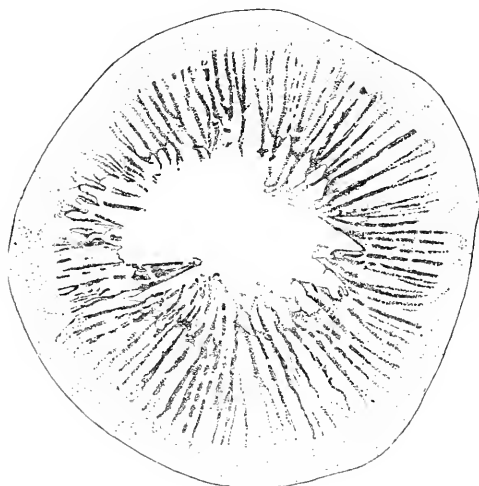


Fig. 106†.

weeks (11 to 20 days in kohlrabi, Hecke) this being the period required for the multiplication of the bacteria in the sub-stomatic chamber and their passage through the intercellular spaces of the epithem into the vessels of the leaf. Generally, however, in artificial inoculations there is a slight darkening of the infected leaf-tooth as early as the sixth to tenth day. For illustrations see Vol. I, figs. 76, 77, 78, 79, 87, 115, 116, 117. These infections were obtained by atomizing upon the plants in inoculation cages (Vol. I, fig. 95) agar cultures diffused in water. Russell placed the bacteria in drops of water extruding from particular water-pores. Hecke tried both methods successfully. The writer's first successes were by plunging leaves into water containing the bacteria and allowing them to remain for some hours. Brenner likewise obtained waterpore infections by this last method, and also by placing the bacteria

\*In fluid collected from water-pores Brenner found the organism multiplied twentyfold in the course of 10 days. Russell also collected several cubic centimeters of fluid from the water-pores, inoculated it with *Bacterium campestris* and made poured plates. The second series of poured plates made 12 hours later "showed many more colonies of the specific germ, thus indicating that the bacteria originally seeded were able to grow in the water."

†Fig. 106.—A small turnip root, showing center rotted out by *Bacterium campestris*. An old plant, but no normal expansion of root. From a field near Baltimore, Md., Sept., 1896. x circa 8.

on particular water-pores which were extruding fluid. Once past the incubation period, the downward progress of the disease is comparatively rapid (1 cm. or more per day), especially if the weather is warm and the soil is moist enough to induce a vigorous growth of the host-plant. The progress of the disease in cool weather and in plants making a slow growth is less rapid (plate 2). On September 7, 1897, during weather very favorable to the progress of the disease, the writer examined a cabbage-plant at Racine, Wisconsin, which bore 170 separate water-pore infections all spreading rapidly. As yet there was no disease of the root, stem, or interior of the well-formed head, nor was there any black stain in the base of any of the petioles. These infections, therefore, probably took place not much earlier than the first of August. Such a plant might be expected to be badly rotted in stem and head in course of another six or eight weeks.

Brenner states that when he inoculated cabbage-plants on a single leaf-tooth many other groups of water-pores subsequently contracted the disease, the organism being presumably carried along the moist surface of the leaf-margin to the other groups of water-pores by capillary attraction. As noted in 1898, the writer saw one large cabbage-plant which bore more than 400 distinct marginal leaf-infections, while many other plants in the same field and in other fields showed from 50 to 150 such separate infections. Sometimes nearly every serrature on a leaf would be infected. The writer has dissected hundreds of cabbage-leaves which were attacked at the time of observation only on their margin, and other hundreds in which the organism was already well into the middle of the leaf or had already entered the stem, as determined by cutting. An extensive marginal infection obtained by spraying, with the entire absence of infection by way of the ordinary stomata is shown in fig. 10. For an early stage of water pore infection see fig. 130a and Vol. I, fig. 87; for a late stage with disorganization, this volume, fig. 11.

The leaf surface of many crucifers is covered with a waxy bloom repellant to water, and this preserves the leaf from injury when submerged for some hours, and also undoubtedly makes it difficult for the bacteria to enter through the ordinary stomata. At least, infections through such stomata have not been observed. Russell's observations and experiments agree with those of the writer.

In case of leaf-infections by way of the water-pores the general progress of the disease is downward in the spiral vessels of the leaf (Vol. I, fig. 76, 77, 78). These are commonly filled densely, many of them at least, by the rapid multiplication of the organism. From the blade of the leaf the bacteria pass into the leaf-traces of the petiole (fig. 109, 110)

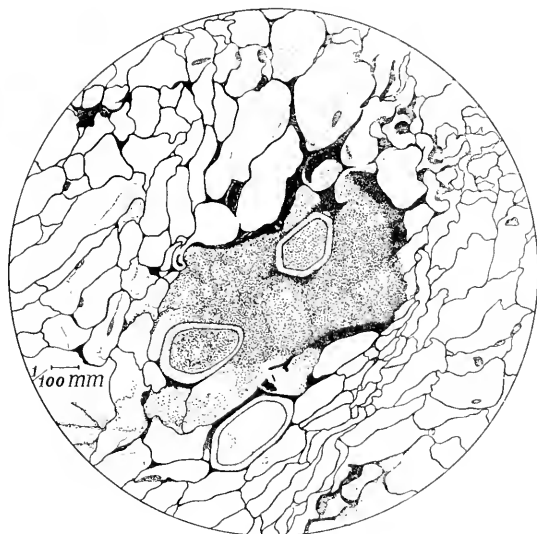


Fig. 107.\*

\*Fig. 107.—Cross-section of a small portion of a blackened kohlrabi bundle, showing cavity due to presence of *Bacterium campestris*. Section from enlarged edible part of plant. Material collected at Miami, Fla., March, 1904. x 500. Slide 293 b 18.

and thence into the stem where, in turn, the vessels of the stem are occluded and browned. Subsequently if the tissues are soft enough the organism passes up and down the stem and out into other leaves, always by way of the vascular system. The rapidity of movement in stems depends on their texture. In hard woody stems the bacteria move with extreme slowness or are entirely hemmed in; in soft juicy stems progress resembles that in the petiole. In leaves which are infected from the stem (fig. 98), the entire leaf-blade may be attacked almost at once and in that case may show signs of wilting. The writer has frequently seen cabbage leaves become flabby, unjoint and fall off while the bacteria were still confined to the petiole, such leaves having been infected by way of the leaf-traces as the result of stem-inoculations. In these cases so many leaf-traces were involved that the leaf was unable to obtain the necessary water-supply. More often some of the leaf-traces are not involved and the leaf manages to function more or less imperfectly for a considerable

time. In such a leaf a part of the veins in the leaf-blade are always blackened considerably in advance of the remainder and wilting may not occur. The writer tried passing 1 per cent eosine water up such petioles by transferring them to the red fluid after cutting them under water. In many cases the eosine only passed up the unobstructed vessels, but whether failure to pass up the bacterially occluded vessels was due simply to the occlusion, or must be ascribed in part to the destruction of the vessel-walls by the bacteria, was not determined.

Whether the first signs on the expanded portion of such leaves are basal or terminal, or on one side or the other of the blade, depends entirely on which leaf-traces are entered first, different ones ramifying to different parts of the leaf (Smith, Hecke). In the end, such leaves are so badly affected that they unjoint and fall from the stem, without, however, any signs of soft rot. It is a slow dry-rot even in turnip-roots. When soft rot or extensive sloughing of the parenchyma intervenes, especially if it begins at the surface, we may at once suspect complications due to the presence of other organisms (see the soft rots). When inoculations are made on the midrib of a leaf, Brenner states that the bacteria pass up-

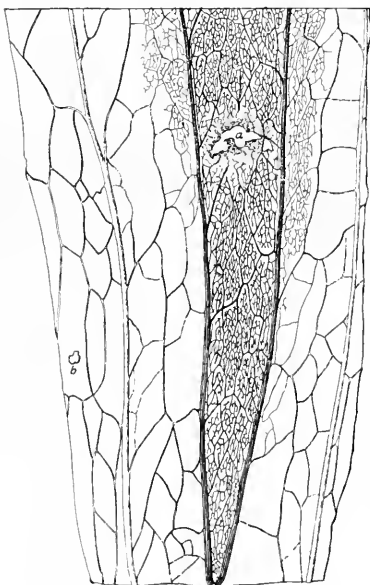


Fig. 108.\*

ward faster than downward. The writer recorded the same fact for inoculated cabbages in 1897, and observed it again particularly in 1906. The writer has frequently observed the inoculated side of the plant to become diseased almost to the exclusion of the other side, but has observed nothing suggestive of the rapid transportation of the bacteria for long distances in a liquid moving stream such as we sometimes conceive to be present in the vessels of a plant. In a plant inoculated on the stem under the fourth leaf Brenner observed the fourth, fifth, and seventh leaves, which were on the inoculated side of the stem, to contract the disease sooner than the sixth leaf, which was on the opposite side of the stem (see cucumber wilt, p. 219). Brenner endeavored to force stained bacteria up cabbage-

\*Fig. 108. — Small portion of a cabbage-leaf near margin, showing how black venation due to *Bacterium campestris* is frequently restricted for a time to angular areas formed by larger veins. This infection may have started from an insect bite at *a*, or may have run in from a group of water-pores; at *b*, is another insect bite. Specimen from a cabbage field in Western New York. Drawn from a photograph. About natural size.

petioles, but neither by suction nor by pressure with mercury could he get them higher than about 2 cm. The stain passed farther but not the bacteria. According to Hecke the bacterial infection passed upward in certain inoculated kohlrabi leaves 5 cm. in the time required to pass downward 2.5 cm. This more rapid upward movement is attributed by him to the effect of the transpiration stream.

Weather conditions favorable to rapid growth are also favorable to the spread of this disease. When two sets of plants are inoculated in the same manner, the one receiving large quantities of water and the other less amounts, the former contract the disease more readily and the organism also passes through the tissues with greater rapidity (Russell).

Varietal resistance to this disease, and the resistance of particular individuals within the variety, are subjects deserving of careful attention. Russell states that "In all probability there is but very little difference in susceptibility, all varieties readily yielding to the disease, if the causal organism is once present." Many cabbage growers think differently, but so far as the writer has had opportunity to examine into the matter himself he has found the statements of particular growers that this or that variety was specially subject based only on isolated observations and easily overthrown by other observations in the same locality or some other. In experiments with kohlrabi Hecke found that the "Vienna Glaskohlrabi," which matures quickly, is less likely to show the disease in the fleshy part as the result of leaf-inoculations, than is the slowly maturing variety known as "Goliath." Generally the farmer thinks that variety most subject which he happens to have planted on infected land, while as a matter of fact on the very next farm the conditions may be reversed. Nothing here said should be construed into a denial of difference in behavior, but only regarded as a reason for caution in drawing conclusions, it being quite in line with what we know of other diseases to suppose there are tendencies to resistance, especially in particular plants, which might be increased and made of economic importance.

According to Russell, cauliflower is the most susceptible plant, while turnips and rutabagas are not very susceptible. The writer found radishes rather resistant.

In North Holland, according to Ritzema Bos, the disease was most prevalent in red cabbage in 1900, but in Savoy cabbage in 1901.

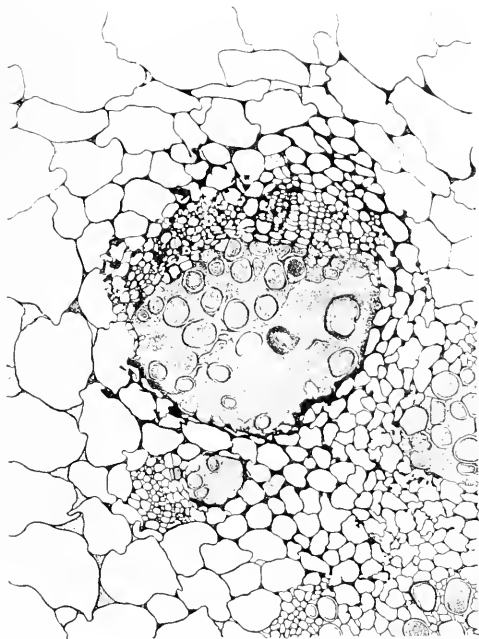


Fig. 109.\*

\*FIG. 109.—Portion of a cauliflower-petiole in cross-section, showing destruction of the xylem portion of several bundles by *Bacterium campestre*. In lower left-hand part of figure bacteria may also be seen wedging apart parenchyma cells. Result of a pure culture inoculation made into blade of leaf by means of needle-pricks. Material fixed in alcohol, infiltrated in paraffin, sections stained with carbol-fuchsin, and drawing made from a photomicrograph.  $\times 100$ . Slide 118-5. For a small portion of this section more highly magnified see fig. 110. For a longitudinal section through a similar cauliflower-petiole see vol. 1, fig. 7.

Since the above paragraphs were written S. F. Edwards has reported (1908) that the Houser cabbage "is practically immune to black-rot under field conditions." Even when pure cultures of the bacteria were inoculated into the cabbage the inoculations were either without result or the disease advanced so slowly as to do but little injury.

The period of incubation is variable. Hecke, inoculating by needle-puncture, obtained the first signs of the disease in from 7 to 28 days on leaves, and in from 9 to 31 days on stems. He made 33 inoculations on kohlrabi leaves by needle-puncture, every one of which was successful; he likewise inoculated 23 kohlrabi plants in the stem by needle-puncture, and

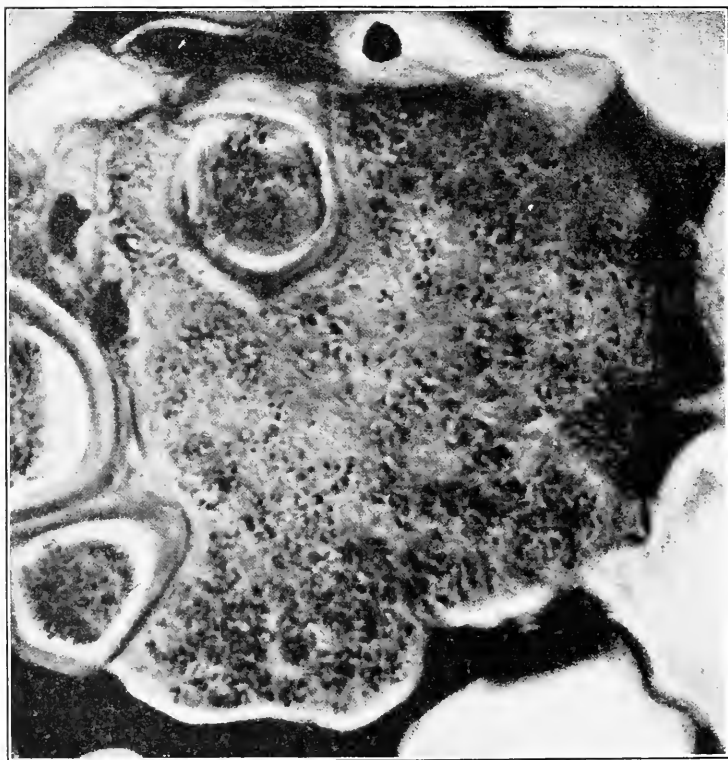


Fig. 110.\*

of the whole number there was not one which did not give some indications of disease. Exclusive of sprays, plunge-experiments, and the use of insects, etc., almost all of my own inoculations were made by needle-puncture without hypodermic injection, and the first distinct signs of disease were generally visible in 14 to 21 days. Brenner also found this period of incubation correct for most of the plants he experimented with. On cotyledons, however, he obtained signs of the disease in 8 days, and the entire plants soon contracted the disease and were destroyed or greatly injured.

\*Fig. 110. Cross-section of a cauliflower-petiole showing bacterial cavity in a small bundle (lower one at left in fig. 109) due to presence of *Bacterium campestris*. From a pure culture inoculation. A paraffin section stained with carbol fuchsin. Enlarged from a photomicrograph.



The greatest contrast to this prompt destruction ever obtained by the writer was on cabbage-plants dwarfed and forced to make a very slow growth by keeping them for a long time in 4-inch pots. These plants, which were inoculated in the autumn, developed the disease, became stunted, and then appeared to grow out of it, but on some of them it reappeared the next summer at the top of the plants in young leaves which were unquestionably infected from the stem by way of the leaf-traces. Fifteen months from the date of inoculation, and more than 30 cm. above the point where the needle entered, the organism was recovered in pure culture from the woody stem of one of these tall spindling plants.

There can be no doubt that the severity of the disease varies with varying seasons. In moist warm seasons the disease often makes a clean sweep on fields which may yield a crop the following season, provided the weather is dry enough to induce slow growth and to prevent wholesale infection by way of the water-pores.

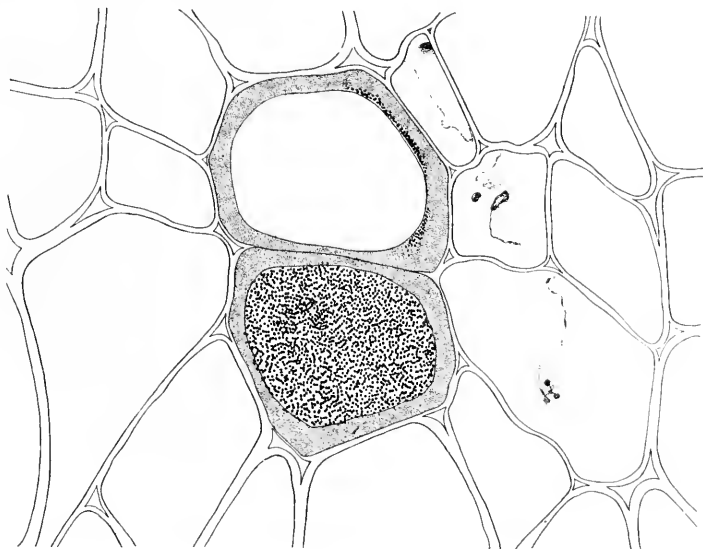


Fig. 111.\*

Owing to its wide distribution, and the ease with which infections may be obtained on a variety of plants, this is a very good disease for the use of classes in schools.

For experimental or demonstration purposes there is a choice in the parts to be inoculated. Young rapidly growing leaves and plants are better than old or slow growing ones, and infection by needle-pricks generally succeeds best when made into the upper fleshy part of stems immediately under leaves or when made into the midrib. Cabbage plants when inoculated on the lower leaves often throw off these leaves before stem-infection has taken place. Brenner notes that when he inoculated into the petiole of the leaf this was frequently thrown off in course of a few weeks. He found inoculations on secondary veins or peripheral veins less successful than those on primary veins.

\*FIG. 111.—Cross-section of root of inoculated turnip plant (No. 53), showing two reticulated vessels, one of which is occupied by *Bacterium campestre* while the other is free (nearly). Surrounding parenchyma cells are free from bacteria. Drawn from a photomicrograph which was made from material fixed in alcohol, infiltrated with paraffin, sectioned on the microtome and stained with carbol-fuchsin. Contents in cells at right are protoplasmic.  $\times 1000$ .

In experiments with *Bacterium campestre* on kohlrabi Hecke found that when the plants were kept excessively moist under bell-jars some parts of the leaf-edge became water-logged (glossy and darker colored) from an excess of water. These parts subsequently died but the plants did not contract the disease from water-pores situated in such suffocated areas. Consult chapter on Angular Leaf-spot of Cotton for similar observations by the writer. The leaves of maize seedlings also frequently become water-logged without contracting Stewart's disease.

*Query*.—Why do the bacteria multiply so abundantly in the vessels?

#### MORBID ANATOMY.

This organism causes no hyperplasias. After it has gained an entrance, which must be ordinarily through parenchymatic tissues (epithem, etc.) the parasite is confined for some time pretty closely, although not exclusively, to the vascular system and even to particular leaf-traces or bundles especially to the spiral and reticulated vessels which are very often filled with incalculable numbers of this organism (figs. 111, 112). When such a state of occlusion exists, especially in juicy parts, the walls of the vessels are destroyed in places (dissolved?) and the organism finds its way into the surrounding parenchyma,

but never or almost never to the surface of the plant. Progress through the parenchyma is slow, apparently on account of its acidity. Often the intercellular spaces are first occupied (figs. 113, 114); the middle lamella is then dissolved (figs. 115, 116) and the elements are separated and squeezed into all sorts of shapes by the multiplication of the bacteria (fig. 117). Subsequently the wall proper of the cell becomes thinner and vaguer and finally seems to disappear altogether. There may be some doubt, however, on the latter point, *i. e.*, as to the final complete solution and disappearance of the cellulose. The dark band at the bottom of fig. 110 is probably formed of compacted cell walls crowded out of the cavity. Similar cell-walls crowded out of the center of the cavity may be seen in fig. 117 as white lines. Lignified tissues are not dissolved although Brenner states that they are.

This statement probably rests on some misinterpretation. The spiral threads and other distinctly lignified portions of the bundle persist. It is the destruction of the surrounding non-lignified tissues which gives rise to the large cavities in turnips and other susceptible tissues.

The formation of cavities by this organism is very common in a number of host-plants (figs. 109, 117, 118 and Vol. I, figs. 6 and 7) and all stages of the separation and destruction of the cells may be studied to good advantage in turnip-roots and cabbage-leaves or cauliflower-leaves, especially the petioles. The occlusion of the vessels and the formation of cavities may also be made out very satisfactorily in kohlrabi and rape (figs. 107, 119). These cavities always begin in the vascular bundles and are occupied by the bacteria in incalculable numbers, scarcely anything like it being observed in the animal kingdom. Small parenchyma-cavities sometimes appear around the smaller blackened veins in leaf-blades, but more conspicuous ones are to be found in the fleshy midrib and petiole. As a whole the parenchyma of the leaf-blade appears to be too dry or too acid for this organism. The pith

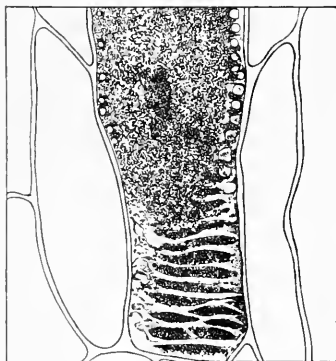


Fig. 112.\*

\*FIG. 112.—*Bacterium campestre* occupying a reticulated vessel in a turnip-root as result of a pure culture inoculation on blades of leaf. Same vessel as shown in fig. 120 but a little farther down. Drawn from a photomicrograph. X 500.

offers a more favorable substratum. Occasionally in cabbage and collards the entire pith of a stem disappears (fig. 105) and in turnips it is not uncommon for cavities in the roots to occupy a large portion of the interior (fig. 106).

Although, as stated, parenchyma is destroyed by the wedging apart of its cells, there are other ways, *i. e.*, it is not infrequent, as shown in fig. 120 and on cross-section in Vol. I, fig. 5, for non-lignified cells surrounding a vessel to be entered and filled by the bacteria rather than to be crushed and crowded out of the way by external multiplication. The cell-wall appears to be intact as shown in the drawings and clearly no great amount of it can be dissolved. It is not easy, therefore, to make out exactly the method of entrance. Probably the bacteria enter these particular cells by way of pits, the vessel being first filled by the organism which then either dissolves the thin separating membrane of the pit or softens and ruptures it.

Harding and Brenner both mention the occasional presence in the bacterial cavities of granules, which do not stain like bacteria, and the origin of which is somewhat doubtful. Hecke states that he found such granules in undestroyed vessels just beyond the advancing margin of the bacterial mass, as determined by serial sections. This substance stained with magdala red but did not retain the iron-haematoxylin. The material was fixed in a mixture of formalin, wood-vinegar and wood-alcohol. Brenner is inclined to consider these granules as in the nature of bassorin and derived from the decomposition of the host-tissues. The writer believes some of them to be dead and more or less disorganized bacteria, which for this reason do not take stains well. Such granules occur in great numbers in sugar-cane attacked by *Bacterium vascularum*. See also Symbiosis, page 111. They offer a good subject for further research.

There are no gaps in the bacterial occupation of particular vessels and, consequently, as Hecke suggests, movement of the organism in the tissues, probably may be assumed to be due to growth rather than to self-motility. The black stain always follows the bacterial occupation, rather than precedes it, but the bacteria are rarely more than 1 or 2 cm. in advance of the pigmentation, so that to a good degree absence of brown stain in the vascular bundles may be taken to denote absence of the bacteria.† This black or brown stain may be located

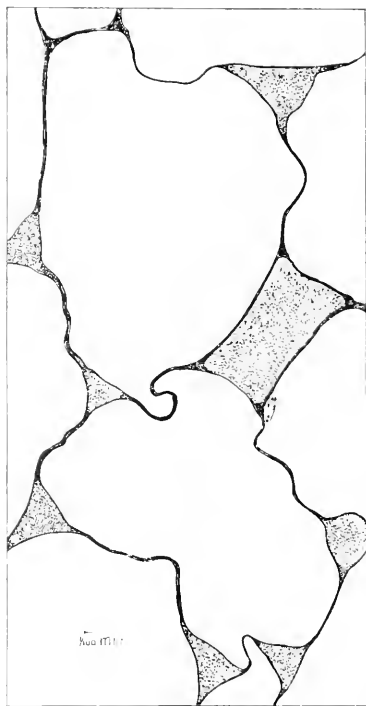


Fig. 113.\*

\*Fig. 113.—Cross-section of small portion of cauliflower-petiole, showing parenchyma to left of fig. 109. Inter-cellular spaces occupied by *Bacterium campestris*. The cells themselves are entirely free. Section stained with carbol fuchsin. Slide 118-5. The bacteria entered this tissue by way of one of the bundles in which there is a large cavity.

†Russell says "the causal organism can frequently be isolated at a point 2 or 3 inches in advance of the darkened tissue." (Bull. 65, p. 23.) This I have not been able to verify and am inclined to think it is a mistake.

Errors may easily occur, since in one of the author's own examinations of leaves infected on the blade no brown stain was detected in a fresh-cut petiole on cross-section, at a certain level, using a good hand-lens, but was plainly visible to the naked eye more than 2 inches farther down (away from the point of inoculation) in a few vessels of one bundle on mashing the tissues for poured plates, and was then detected on the original cross-section, the surface of which had meanwhile become dry and somewhat lighter colored. These browned vessels which contained numerous bacteria, were 11 cm. below the place of inoculation, and 6 cm. below the point where the brown stain in the vessels was at first supposed to have entirely disappeared. The poured plates yielded numerous colonies of *Bacterium campestris*.

between the vessels as shown in plate 17 or may affect the wall of the vessel itself. When located in the vessel the lignified parts absorb the stain.

In many large cabbage stems the writer noted a decided increase in the number of chlorophyll bodies in tissues immediately surrounding the bacterially infested vessels. Frequently the green color was as conspicuous as the brown stain. Without knowing positively, the writer has been inclined to regard this phenomenon as brought about by the osmotic movement of food stuffs toward the bacteria, and by the liberation of unusual amounts of carbon dioxide as a result of the bacterial multiplication. The same phenomenon occurs in the tumor-strands in crown-gall of the daisy (Circular 85, p. 3).

#### THE PARASITE.

As already stated, the cause of this disease is *Bacterium campestre* (Pammel) EFS,\* a yellow one-flagellate Schizomycete (fig. 121). It is sometimes motile when taken directly from the plant and examined in a hanging drop of water, but more often not, especially if it

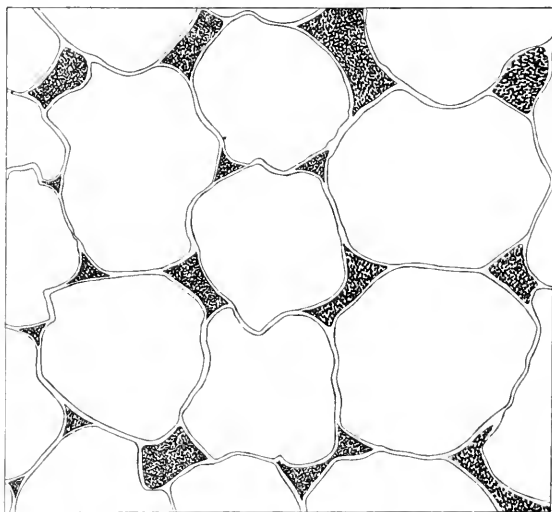


Fig. 114.†

is taken from parts which have been occupied for some time (see *B. tracheiphilus*, pp. 221, 242). It is a rather small and somewhat variable organism, especially in old growths. When crowded in the plant or when taken from old cultures, it often much resembles a coccus, *i. e.*, it is a very short rod with rounded ends (Vol. I, fig. 18). When multiplying rapidly in the plant or when taken from young cultures it is a rod usually one and a half to four times as long as broad (figs. 122, 123). In such situations it occurs singly or more often joined in pairs, end to end, and usually with a distinct constriction; in some media short chains of

\*Syn. *Bacillus campestris* Pammel, *Pseudomonas campestris* (Pammel) EFS. The nomenclature of the bacteria in this volume corresponds to my ideas on this subject as enunciated in Vol. I, to which the reader is referred. Potter writes *Bacillus campestris rutabaga*.

†Fig. 114. — *Bacterium campestre* occupying the intercellular spaces in the parenchyma of a turnip-root. Cross-section of inoculated plant (No. 53). Protoplasmic contents of cells omitted. The bacteria have crowded the cells apart somewhat, but are still confined to the intercellular spaces. A continuance of this multiplication and crowding for a few weeks would result in the collapse of the cells and the formation of a cavity such as that shown in fig. 117. Drawn from a photomicrograph. × 475.

four or more elements occur. Single elements then generally fall within the following measurements:  $0.7$  to  $3.0\mu$  by  $0.4$  to  $0.5\mu$ . It is often somewhat irregular in shape, *viz.*, slightly crooked or larger at one end than at the other (figs. 123, 124). The appearance of the organism from a young bouillon culture at  $30^{\circ}\text{C}$ . and from old potato cultures at refrigerator temperatures, is shown in figs. 125-127. When treated with flagella-stains the diameter is greater, *viz.*,  $0.7$  to  $0.9\mu$ , or thereabouts. It has no distinct capsule (Harding). In sugar-rich media the organism, like other species of this genus, frequently grows out into long chains or into filaments in which no septa can be detected (Vol. I, fig. 19). These may be  $50$  to  $100\mu$  long. Brenner figures a very short form which he designates as "the normal form on old agar cultures," and chains from a "very old exhausted culture." He also states that he sometimes found in the plant, in parts long diseased, rods, much longer than the ordinary short form. Hecke figures the organism as a short rod; Harding, as a short rod and as chains composed of a half dozen easily distinguishable segments. Harding says the cells

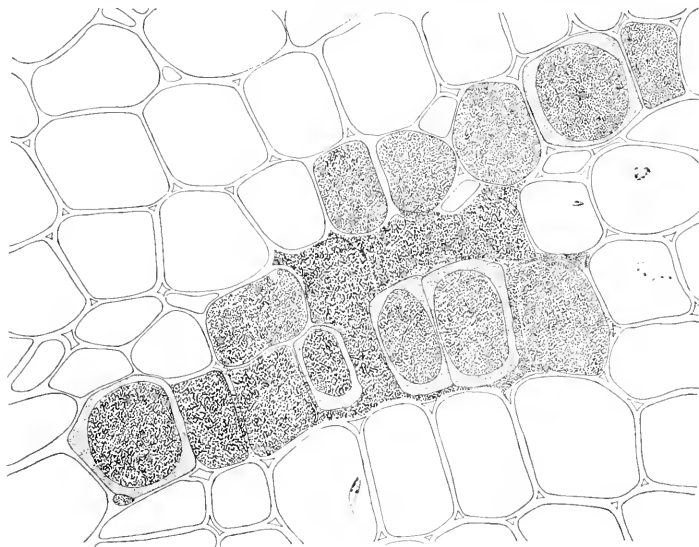


Fig. 115.\*

are usually isolated, but during rapid growth short chains consisting of 2 to 8 segments are formed. Under exceptional circumstances, not well understood, Harding observed long filaments during the first 24 hours of growth. Pammel figures it as a rod 2 to 4 times as long as broad, occurring singly, or in pairs or three's joined at the ends and with a plain constriction. In old exhausted cultures Brenner observed rods with polar bodies. Harding frequently obtained a dense polar stain and feeble central stain when he used Ziehl's carbol-fuchsin. The writer has also seen this. Pseudozoogloecae occur frequently in various culture-media. No spores have been observed, but under certain conditions the organism is very resistant to drying. The flagellum is several times the length of the body and arises at or near the end. The writer has not observed more than one flagellum on a pole. The motions of the organism consist of tumbling, sinuous, and darting movements.

\*FIG. 115.—Cross-section of a turnip-root (plant No. 53), showing commencement of a cavity due to *Bacterium campestris*. Nuclei are visible in several cells. Cellulose walls clear; lignified walls (vessels) dotted. Drawn from a photomicrograph.  $\times 500$ .

The organism is wax-yellow, deeper or paler according to circumstances, changing to a dirty yellow brown in the plant and in certain old cultures. The color on coconut flesh standing in distilled water is approximately Ridgway's Naples yellow. On turnip cylinders

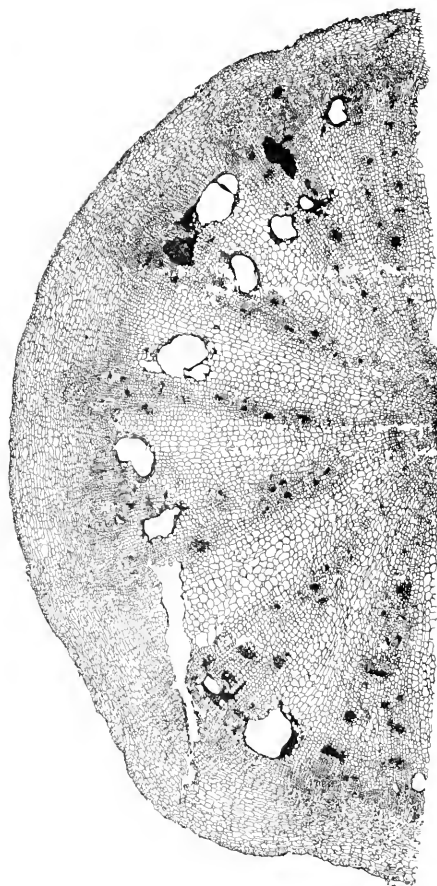


Fig. 118.†

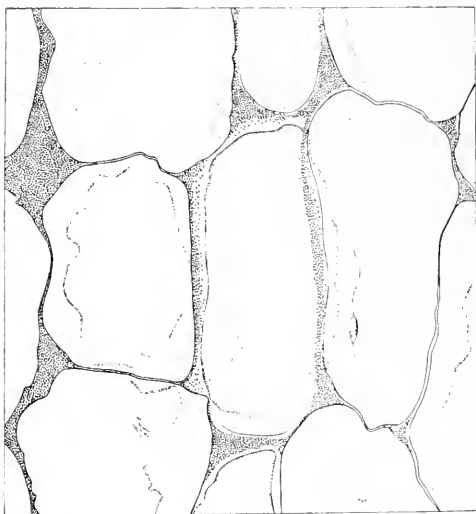


Fig. 116.\*

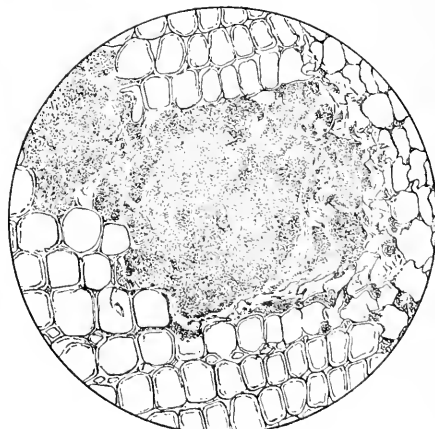


Fig. 117.‡

\*Fig. 116.—Longitudinal section of a turnip-root, showing how intercellular spaces are occupied and parenchyma cells wedged apart by *Bacterium campestre*. A later stage than fig. 114. Drawn from a photomicrograph.  $\times 475$ .

‡Fig. 117.—Bacterial cavity in interior of a turnip-root (plant No. 53), due to *Bacterium campestre*, which was inoculated by needle-pricks on blades of two leaves 52 days prior to fixing material. Exterior sound. Slide 115-I. Drawn from a photomicrograph.

†Fig. 118.—Cross-section of a small turnip-root, showing bacterial pockets and wide distribution of organism in vascular system. Inked from a photomicrograph. In a cross-section of this root lower down the writer counted 146 bundles occupied by masses of the bacteria and separated by unoccupied parenchyma. Surface of root unbroken.

steamed in water the formation of the brown pigment was very decided, finally approximating bistre or mummy brown. Harding observed a similar prompt browning in cultures made on white winter radish and on cauliflower. Non-cruciferous substrata so far as tested do not give any such deep brown pigmentation.

Colonies of this organism on agar or gelatin are smooth, wet-shining, pale yellow, round, flat to convex (Harding), thinning out to a distinct entire margin (fig. 128). The surface colonies at first are often quite pale, becoming yellower with age. Buried colonies do not develop speedily, neither is the surface growth very rapid; Harding says—rapid at 28° C. Feathery X-shaped crystals of ammonium magnesium phosphate are formed after some days in cultures on peptonized beef-broth-agar (fig. 128). On +15 nutrient agar after a time a white chemical halo forms around the colony, streak, or "nail head" of the stab. This appears to be common, however, to various species of *Bacterium*. So also are the crystals.

On meat-extract-peptone gelatin, Hecke describes the young colonies as small, cloudy, colorless, circular drops which became plainly yellow with age and feebly zoned concentrically, the gelatin liquefying slowly. At his room temperature (15° C. ?) the growth on this medium was slow, the surface colonies on a plate 15 days old measuring only about 3 mm. in diameter in a liquefied zone 7 mm. in diameter. On neutralized kohlrabi-gelatin similar results were obtained. On the contrary, in non-neutralized kohlrabi-gelatin growth was extraordinarily slow, so that the colonies were not visible until after the tenth day.

In my +10 nutrient gelatin, in rather thin sowings in Petri-dish poured-plates, at the end of 7 days, at 10° to 20° C., the surface colonies of *Bacterium campestre* under the Zeiss 16 mm. objective and 12 ocular were small, circular, and homogeneous (fine granular), with entire margins; the buried colonies were *globosc-lobulated* and less than 1 mm. in diameter.

*Bacterium campestre* isolated by the writer from a cabbage leaf in June, 1908, on a thin-sown agar plate (10 colonies) gave thin, flat, pale yellow, circular surface colonies with a slight tendency to rings but no other distinct naked-eye structure. At the end of 4 days at 28° to 30° C., the largest colonies were 8 to 10 mm. in diameter, the surface smooth and wet-shining. With Zeiss 16 mm. apo., and 12 ocular the margin of the colonies showed no special characteristics. Under this magnification the colonies were uniformly fine granular except the extreme margin which was a little paler and nearly amorphous in structure. In a plate of the same lot containing about 100 colonies, the surface colonies on the fourth day

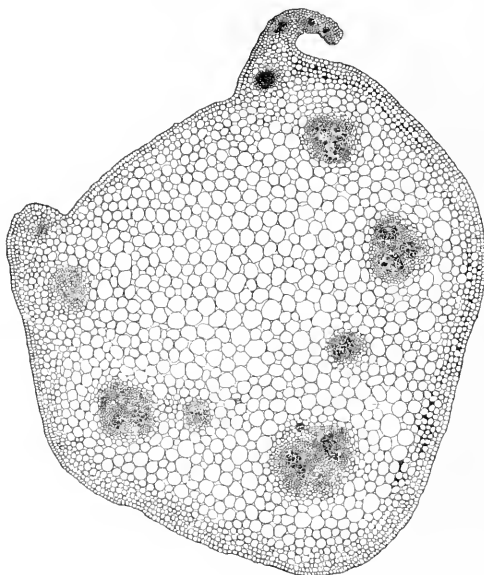


Fig. 119.\*

\*FIG. 119.—Cross-section of a rape petiole attacked by *Bacterium campestre*. Plant inoculated Dec. 19, 1896. Leaf fixed in alcohol Jan. 9, 1897. Bacteria restricted to the bundles, i. e., to heavily shaded parts, except on right side near periphery where intercellular (shaded) spaces are occupied. Drawn from a photomicrograph made with a planar lens, from a stained microtome (paraffin infiltrated) section. Actual diameter about 2 mm. Slide 107 A.

were 4 to 7 mm. in diameter and a few of them had fused. These colonies were circular, smooth, flat, and distinct on the margin. The buried colonies were *small* and elliptical. The agar was that ordinarily used in the laboratory (see Vol. I, p. 195).

Under low powers of the microscope Harding found colonies on standard nutrient agar plates to consist usually of a central circular area homogeneous in texture and embracing a darker spot as nucleus. This portion of the colony was surrounded by a coarsely granular zone usually darker in color than the center. The periphery of the colony formed a third zone either homogeneous or very finely granular.

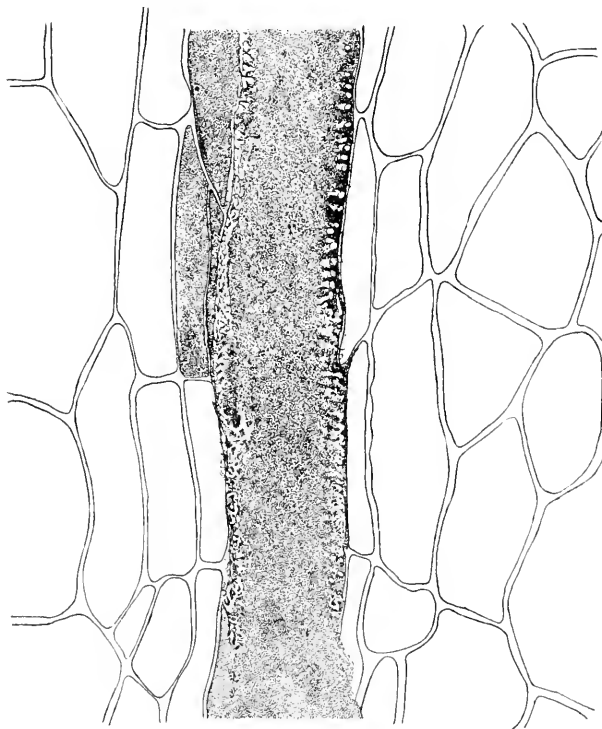


Fig. 120.\*

The surface of old cultures on agar, potato, etc., frequently becomes a substratum for the development of new colonies. These are usually not very large and are generally circular in outline. They are readily distinguished from the older growth by a difference in color, which gives to the surface a spotted appearance.

Growth in stab cultures is always or nearly always best near the surface. In streak cultures the surface growth is smooth and wet-shining.

It is an organism easy to isolate and cultivate, growing readily on a variety of media. The organism is not specially sensitive to dry air or to its own decomposition products.

\*FIG. 120. --Longitudinal section through root of a turnip-plant (No. 53) parasitized by *Bacterium campestris*. The section passes through a reticulated vessel. Three non-lignified cells to the left of the vessel are also occupied by the bacteria; the rest of the tissue is entirely free. Drawn from a photomicrograph. Circa  $\times 500$ .



It prefers neutral or alkaline media, and its growth is inhibited or retarded by acids (strong litmus reaction). No true pellicle is formed on neutral peptonized beef-bouillon (Harding) but after a time there is a yellowish ring. It grows very copiously on steamed potato, standing in water; the yellow slime soon fills the fluid (Vide C. f. B., 2 Abt., 111 Bd., Tafel vi, fig. 4), converting it into a solid alkaline mass which turns brownish with age. It destroys potato-starch so that it will not react with iodine. The conversion of the potato-starch, when potato-cylinders are used as the culture-medium, is never quite completed. There always remain some scattering starch-grains or groups of grains which react purple with iodine, although on first mashing in the iodine water, which should be abundant,\* all appear to have been converted. The conversion is so nearly complete, however, that one might probably safely estimate it at 99 + per cent, not only for this organism but also for several similar yellow species, e. g., *Bacterium phascoli* (plate 17, fig. 3). The action was always well marked and often practically complete after two weeks (Harding). Potato cultures are of a butyrous consistency and give off an odor of ammonia (Harding).



Fig. 121.†

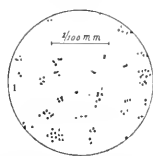


Fig. 122.‡

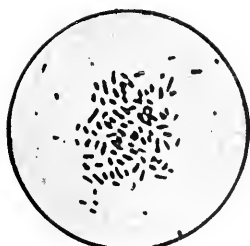


Fig. 124.||

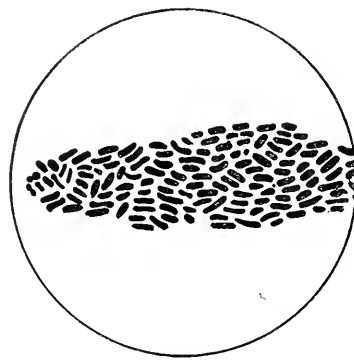


Fig. 123.§

The organism blues litmus milk and throws down the casein slowly by means of a lab ferment. The milk is not coagulated into a stiff mass but remains fluid for a long time, the small amount of clear whey slowly increasing over a mobile, bulky precipitate, which gradually becomes compacted. At no time is any acid developed in litmus milk. Harding also observed the slowly increasing layer of whey on top of the culture in milk and says that the casein is gradually digested, the liquid then assuming a yellowish tint. His time limit for beginning of the extrusion of whey is 3 to 10 days. Tyrosin crystals have been observed. It inverts cane-sugar (?). It liquefies gelatin and Loeffler's blood serum, both slowly.

\*Ammonia bleaches alcohol iodine and if only a little of the latter is added there may be enough ammonia in the culture to interfere with the test, if the particular organism on trial produces this alkali abundantly.

†Fig. 121.—*Bacterium campestre*, from a cover-glass preparation stained with Fischer's modification of Loeffler's flagella stain. Feb. 27, 1897. Cover made from an agar culture 8 days old which contained many actively motile rods (tube 4, Feb. 18, descended from a colony isolated from a turnip). Drawn directly from the slide. x 1000.

‡Fig. 122.—*Bacterium campestre*: Cover-glass preparation direct from stem of charlock (*Brassica sinapistrum*) Racine, Wisconsin, Aug. 30, 1897. Drawn with a Zeiss 12 ocular and 2 mm. apochromatic oil immersion objective 1.30 n. a.

§Fig. 123.—Contact preparation of *Bacterium campestre* from a gelatin-plate culture. Organism isolated from kohlrabi and stained with fuchsin. x 2600. After Hecke.

||Fig. 124.—*Bacterium campestre*: Cover-glass smear preparation from a cabbage-stem, stained with carbol fuchsin. Photomicrograph x 2000.

In gelatin-stab-cultures liquefaction begins at the surface and passes to the walls of the tube and thence horizontally downward more and more slowly, the depths of the gelatin, even along the track of the needle, remaining solid for a long time (fig. 129). The liquefied gelatin may be cloudy at first as in the middle tube but is finally clear unless shaken. The writer observed some differences in the rate of liquefaction, these depending on the kind of gelatin medium used. Occasionally in unfavorable gelatins there was no liquefaction. On peptonized beef-broth-gelatin feebly acid to litmus, growth was feeble. In the same gelatin rendered more alkaline (feebly alkaline to litmus) growth was better. In stab-cultures in the latter, liquefaction began in 24 hours and was completed (10 cc.) in 15 days at 17° to 19° C. A much longer time than this is often required for complete liquefaction—two months or more. In the writer's experiments 0 gelatin was liquefied more rapidly than +20 or -20 gelatin. According to Harding liquefaction begins in 3 to 18 days.

In streak-cultures on Loeffler's blood-serum at the end of 20 days at about 23° C. there was an abundant dull yellow growth, and a slow liquefaction. All the upper part of the slant was fluid or transparent. Only the extreme base of the serum under the V had retained its original opaque-white color. The fluid serum was pale brownish by reflected light, and the stain and transparency passed down into the solid part. In comparison with a streak



Fig. 125.\*

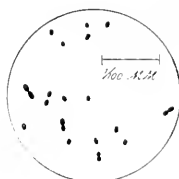


Fig. 126.†

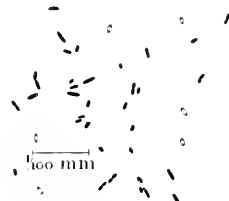


Fig. 127.‡

of *Bact. phaseoli* of the same age *Bact. campestre* showed more liquefaction and stain, but not more growth: By reflected light the contrast in color of the serum, white vs. brownish, was decided (tests of May, 1909). Subsequently this contrast became less.

It is an organism rather sensitive to acids, even those derived from plants. Complete data (quantitative) are not available. Further experiments should be made. According to Harding the vitality of the organism is lessened by long cultivation, *i. e.*, it liquefies gelatin more slowly and is less resistant to heat and to desiccation. It destroys the middle lamella of cell-walls and possibly(?) on prolonged action the cellulose of crucifers, but not lignified tissues. It has no solvent action on Swedish filter-paper. It produces indol slowly in sugar-free peptonized beef-bouillon or peptonized Ushinsky's solution; it does not reduce potassium nitrate to nitrite when grown in peptonized bouillon or with cane-sugar in Fischer's solution. The organism had no characteristic odor, except (Harding) the strong odor of crucifers when grown on these substrata and in bouillon an odor of sweet corn.

\*FIG. 125.—*Bacterium campestre* from a potato culture kept 4 months in ice-box at 12° C. Fluid at bottom of culture was filled solid with bacteria, but the growth was still a fresh yellow color. Drawn unstained from a hanging drop with 2 mm. objective, 1.30 n. a., and 12 compensating ocular, Mar. 8, 1905.

†FIG. 126.—*Bacterium campestre*, drawn unstained from a hanging drop Mar. 9, 1905, after 48 hours in beef bouillon at 30° C. The organism was actively motile and short; termo-like paired rods were the common form. No long rods were observed. Bouillon was thinly clouded. It was made from a typical culture on potato, *i. e.*, that which furnished material for fig. 125.

‡FIG. 127.—*Bacterium campestre*, from a very old potato culture (brownish yellow slime stirred up in water) made Mar. 8, 1905, from a culture inoculated Oct. 4, 1904, and kept in a refrigerator a long time at 12° C. A larger proportion of the rods were vacuolate than are here shown.

So far as known it is strictly aerobic, *i. e.*, it does not produce gas or grow in the closed end of fermentation-tubes in peptone-water or peptonized beef-bouillon with any of the following carbon compounds: grape-sugar, fruit-sugar, cane-sugar, galactose, milk-sugar, maltose, dextrin, mannit, glycerin; neither will it grow in the closed end in potato-broth, cabbage-broth, or cauliflower-broth; nor with nitrates (Harding). If any acids are produced, the pres-

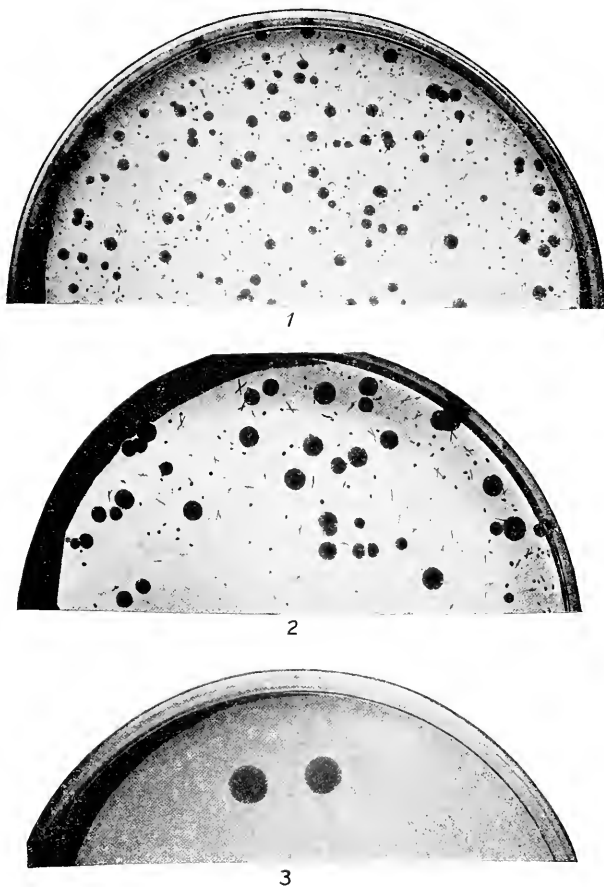


Fig. 128.\*

ence of air is required and they are readily obscured by the production of alkali (ammonia). It is not conspicuous as a reducer of litmus. Its reducing powers are variable. Occasionally some hydrogen sulphide is formed. In cabbage-broth containing litmus the organism

\*FIG. 128.—Petri-dish cultures of *Bacterium campestre*, showing character of colonies and effect of crowding on size. Cultures 8 days old at room temperature. Figs. 1 and 2 contain crystals due to growth of organism in +15 agar. Small dots are buried colonies; medium-sized faint colonies, as in the center of 2, are thin expansions of the same organism between agar and bottom of dish. These poured-plates were made directly from blackened vascular ring of a young shoot of collard shown in fig. 105 (at the point marked x). Natural size.

reduced the litmus in course of a few days, except at the top. On some vegetable media, and with various sugars, a feebly acid reaction was sometimes detected, but the nature of the acid is unknown. Possibly sometimes the writer may have had contamination in his cultures, since Harding has found in some of his cultures a contaminating organism having the group number 211.2223532. The subject is open to further study in which connection the interesting pages 31-33 of Harding's paper (1910) should be consulted. It does not grow luxuriantly in Fermi's solution, Uschinsky's solution, or Cohn's solution, usually it does not grow at all in the latter and when it does there is no fluorescence. In Fermi's solution after 2 weeks there was thin clouding, no pellicle, and a scanty pale precipitate. It will not grow in an atmosphere of hydrogen, nitrogen, or carbon dioxide. In vacuo it also grows feebly in proportion to the completeness of the exhaustion of the air. It will not grow in peptonized beef-broth to which chloroform has been added. This experiment was repeated in February 1906, with the same result (fig. 130). Four tubes inoculated February 12 remained entirely free from clouding (February 24). It is promptly killed in agar plates by direct sunlight (30 minutes or less).

It produces a brown pigment soluble in water, and a yellow pigment insoluble in water but soluble in glycerin, ethyl alcohol, methyl alcohol, acetone, ammonium carbonate, or glacial acetic acid. This yellow pigment appears to be associated with a fat, *i. e.*, it is a lipochrome. Harding states that the yellow pigment is soluble in ethyl and methyl alcohol, is unchanged in glycerin, and is darkened in carbon-bisulphide, xylene, gasoline and chloroform. He found it slowly destroyed in dilute acetic acid, and destroyed in sulphuric ether, dilute hydrochloric acid, sulphuric acid, and nitric acid. The writer found the yellow pigment bleached by contact with carbon-bisulphide, xylol, toluol, ether and chloroform. The color is lodged in the organism itself. The brown pigment is not formed in beef-broth, or in peptone-water with grape-sugar.

The minimum temperature for growth is  $5^{\circ}\text{C}$ . or thereabouts. Its optimum temperature is  $30^{\circ}\text{C}$ . or thereabouts. Its maximum temperature is  $38^{\circ}$  to  $39^{\circ}\text{C}$ . The thermal death-point is  $51^{\circ} + \text{C}^*$ .

It tolerates sodium hydrate in peptonized beef-bouillon up to  $-40$ , and plant acids up to  $+30$  or  $+40$  (?).

Young cultures stain readily with various basic anilin dyes. Harding usually obtained a polar stain with Ziehl's carbol-fuchsin. In agar cultures 20 days old he says many of the individuals stain feebly with methylene blue unless it is heated or applied for a long time. In sections of the tissues the organism stains very satisfactorily with Ziehl's carbol-fuchsin (3 to 5 minutes' exposure), with nigrosin, and with Heidenhain's iron haematoxylin. Hecke reports better success with Benda's iron haematoxylin than with carbol-fuchsin, *i. e.*, clearer sections. With this stain, by proper differentiation, it is possible to obtain fine contrasts, *i. e.*, the bacteria remain black on a pale background. He used weak acetic acid after carbol-fuchsin for differentiating. Good contrasts may be secured also by a suitable contrast stain, *e. g.*, methyl green (2%) in water 18 hours; solid green, sat. water sol., 1 minute.

Brenner states that the organism grows readily in Fischer's nutrient mineral solution† with cane-sugar for the carbon food and nitrate of potash as the only nitrogen food. In other words, following Fischer's classification, it is nitrobacterium. It also grows well, according to Brenner, in Fischer's nutrient mineral solution with addition of grape-sugar and asparagin or ammonium tartrate as the nitrogen food. It grew moderately in the same

\*Harding reports great variations in the thermal death-point, depending on age of culture, length of time grown on artificial media and temperature at which culture was grown. His highest thermal death-point is  $32^{\circ}\text{C}$ ., the lowest  $14^{\circ}\text{C}$ . For the writer's method of making thermal death point tests see Vol. I, page 77.

	p. ct.
†Distilled water.....	100.00
Dipotassium phosphate.....	1.00
Magnesium sulphate.....	0.20
Calcium chloride.....	0.01

mineral solution with cane-sugar and ammonium chloride as the nitrogen food. It grew feebly in these media when glycerin was substituted for the sugars, and not at all in the potassium nitrate solution when glycerin was substituted for cane-sugar.

These statements are perhaps open to criticism owing to the difficulty of obtaining cane-sugar and mineral salts entirely free from organic nitrogen. If in the presence of air this organism can use glycerin as a carbon food and if it can take nitrogen from potassium nitrate it is difficult to understand why it did not grow when glycerin was substituted for the cane-sugar. Brenner offers no explanation. The writer's interpretation is that the organism obtained its nitrogen not from the potassium nitrate, as Brenner supposed, but from some unsuspected, slight impurity in his cane-sugar and consequently when glycerin was substituted growth could not take place because there was no available nitrogen food. A further reason for this conclusion is that I have repeated Brenner's experiments, using chemicals supposed to be pure, but certainly not entirely free from extraneous substances, and have obtained somewhat contradictory results. Moreover, a very slight, but distinct growth was obtained in twice distilled water, to which only the mineral elements of Fischer's solution had been added, to wit: Dipotassium phosphate, magnesium sulphate, and calcium chloride. A decidedly better growth was obtained by adding cane-sugar, but this growth was not increased or only slightly increased by the further addition of potassium nitrate. The incomplete culture medium (supposed to be free from nitrogen but probably not altogether free) gave as good results, or nearly as good (clouding and bacterial precipitate), as the one made complete by the addition of potassium nitrate. A third objection lies in the fact that the organism does not reduce nitrates to nitrites or to nitrogen, so far as can be determined by the starch-iodin sulphuric acid test.

This experiment was repeated again in 1909 using for the potassium nitrate Merck's guaranteed reagent, with the following results:

Notes of July 30, 1909, on inoculations of June 29, which were by 2 mm. loops from young bouillon-cultures into 10 cc. portions in very clean test-tubes of resistant glass.

- (1) Fischer's mineral solution consisting of distilled water, dipotassium phosphate, magnesium sulphate and calcium chloride.

Fluid cleared, slight yellow precipitate. Earlier, *i. e.*, during the first 2 or 3 weeks, there was a feeble clouding showing presence of N. and C. in the medium.

- (2) Fischer's mineral solution plus ordinary white cane-sugar.

Fluid nearly cleared. A very slight distinctly yellow precipitate—same as No. 1. Earlier the fluid was feebly clouded.

- (3) Fischer's mineral solution plus c. p. cane-sugar.

Fluid cleared. Slight yellow precipitate. Closely like 1 and 2. During the first weeks there was the same amount of very thin clouding. The change in sugar made no change in the behavior.

- (4) The same as No. 2 plus 1 per cent  $\text{KNO}_3$  (Merck's G. R.).

Thinly clouded, some pseudozoogloecae. A small amount of yellow precipitate. Distinctly more growths than in Nos. 1, 2, or 3.

- (5) The same as No. 3 plus 1 per cent  $\text{KNO}_3$  (Merck's G. R.).

Like No. 4 in clouding, etc. There is a scanty precipitate without much yellow in it. More growth than in Nos. 1 to 3.



Fig. 129.\*

\*Fig. 129.—Stab-culture of *Bacterium campestre* in nutrient gelatin o to phenolphthalein, after 12, 28 and 46 days at about 23° C. Only upper part has liquefied. Lower part has not liquefied even along needle-track where some growth has taken place. In lower tube there is a heavy yellow precipitate at bottom of liquefied clear gelatin. Inoculated June 5, 1897.

*Results.*—There has been two or three times as much growth in tubes to which the potassium nitrate was added, but it is not a good growth, *i. e.*, such as would take place in bouillon with peptone. Tests made some days ago, *i. e.*, after a distinct difference developed, showed no nitrite present in inoculated tubes containing the potassium nitrate, so the puzzle is where the bacterium obtains its necessary nitrogen. Perhaps under stress it is able to assimilate N. slowly from unsuitable material just as *Bact. hyacinthi* under similar conditions is able to take C. from potato-starch. On July 16, for comparison, inoculations were made in Fischer's mineral solution plus 1 per cent cane-sugar and 1 per cent Witte's peptone. These tubes although they have not been inoculated as long as the ones containing potassium nitrate have given twenty times as much growth (clouding and yellow precipitate).

Further tests were made in February, 1911, as follows: To Fischer's nutrient mineral solution 1 per cent nitrate of potash was added. The strained solution was then divided into two equal portions. To one half was added 1 per cent cane-sugar and to the other 1 per cent glycerin. Inoculations were made from young bouillon cultures, and for checks on the amount of growth additional inoculations were made into our ordinary +15 peptonized beef bouillon. Two strains of the organism were used, one isolated in my laboratory, the other received from Harding of Geneva, N. Y. The results after 26 days were as follows:

(1) N. Y. strain in Fischer's nutrient mineral solution with cane-sugar and potassium nitrate: Fluid clear, moderate pale yellow precipitate and interrupted white pellicle shaking down easily into small fragments which make the fluid very flocculent. Five tubes: About  $\frac{1}{3}$  as much growth as in the check-tubes in peptone beef bouillon which have a yellow rim and a copious yellow pellicle.

(2) Washington strain in the same: Less growth, scanty pale rim, no pellicle. Scanty yellow precipitate, no flocculence on shaking, but a moderate clouding. Five tubes: About  $\frac{1}{3}$  as much growth as in the checks in the peptone bouillon.

(3) N. Y. strain in Fischer's solution with Schering's c. p. glycerin and potassium nitrate: A distinct growth but less pellicle and less precipitate than in 1. A thin rim with no distinct color. Fluid well clouded on shaking with numerous small flocculent masses. Five tubes: About one-tenth as much growth as in the peptone bouillon checks.

(4) Washington strain in same: Like 3 but more thinly clouded. Yellow precipitate. Five tubes: About one-fifteenth as much growth as with checks. Each tube was tested with boiled starch water. 1:200 potassium iodide water and a few drops of sulphuric acid water. No blue reaction took place.

I can not think, therefore, that this organism is a nitrobacterium in Dr. Fischer's sense of the word, since it does not reduce nitrates to nitrites, so far as can be determined by the test with starch, potassium iodide, and sulphuric acid, and obtains its nitrogen much more readily from peptone than from  $\text{KNO}_3$ . It can use glycerine in presence of  $\text{KNO}_3$ .

Hecke isolated this bacterium in the winter and in spring from frozen kohlrabi. The organism probably winters over in the soil, but up to this time no one has actually played it out of soils. In Smith and Swingle's experiments ten freezings and thawings in course of about 6 hours did not destroy all of the individuals in a bouillon culture, but the first two or three freezings destroyed most of them.

In experiments made some years ago, the writer found this organism much more resistant to dry air than Harding's first report would indicate, to-wit, in Harding's experiments invariably destroyed in 45 hours, and 7 out of 8 cover-slips sterile at the end of 21 hours. In my own tests the organism on 8 out of 24 cover-slips was alive after 34 days when inoculated from a potato-culture 2 days old, and on 2 out of 23 cover-slips when inoculated from bouillon. On agar the writer found the organism very resistant, to-wit, alive after  $17\frac{1}{2}$  months. Much seems to depend on the thinness of the layer and the nature of the surface on which it is dried. Recently Harding and his associates have shown that when placed on cabbage seed and set away in test tubes, sealed and unsealed, a certain number of the bacteria were still living at the end of a year.

Harding tested resistance to disinfectants by adding one drop of a freshly clouded bouillon-culture to 10 cc. of the substance and making bouillon sub-cultures therefrom at

the end of 1, 2, 5, 10, and 15 minutes. Lysol in 0.5 per cent solution killed in 1 minute, but in 0.25 per cent solution it failed to kill in 15 minutes. Carbolic acid in 0.625 per cent solution killed in 5 minutes, but not in 2 minutes.

The organism is quite sensitive to the presence of sodium chloride, but not as much so as *Bacterium phaeocoli*. It grew very feebly in salted gelatin (0 gelatin converted into +26 by adding c.p. HCl). Further comparisons should be made.

The organism is able to live in mixed cultures for a considerable time (Russell, Smith).

In agar cultures at room-temperatures Harding states that the organism remained alive from 4 to 6 months. In the cool-box on potato the writer has kept it alive for a year (average temperature about 12° C.).

Harding injected fresh bouillon cultures (2.5 cc. subcutaneously, 2 cc. intravenously, and 4 cc. subperitoneally) into rabbits with no ill effect other than a temporary loss in weight.

#### RÉSUMÉ OF SALIENT CHARACTERS.

##### POSITIVE.

Pathogenic to Cruciferae, dissolves middle lamella, plugs vessels, forms numerous closed cavities in the host-plant; in cabbage causes conspicuous black stain in veins of leaf. Short rods with rounded ends, single or in pairs, and occasionally in short chains of 4 or more; sometimes much resembling a coccus (*Coccobacillus*) when crowded in the plant or in old cultures; sometimes slightly curved or irregular in shape. Long chains or non-septate filaments frequently occur in sugar-rich media. Pseudozoogloae; yellow on all media, changing to dirty yellow-brown in the plant and on cruciferous substrata (culture media); motile in newly diseased tissues and in young cultures, 1-flagellate; very resistant to drying under certain conditions.

Surface colonies on agar or gelatin rather slow-growing, circular, pale yellow at first, deepening with age, smooth, wet-shining, flat, with distinct margin; buried colonies small and slow-growing; feathery X-shaped crystals of ammonium magnesium phosphate formed in beef-agar after some days; white chemical halo on nutrient agar; gelatin and Loeffler's blood serum liquefied slowly with brownish stain; colonies on gelatin feebly zoned concentrically. Rate of liquefaction depends upon the medium, it may sometimes begin in 24 hours in peptonized beef-broth gelatin (feebly alkaline to litmus) and be completed in 15 days, often slow.

Growth in stab-cultures is usually best near the surface. Neutral or alkaline media produce the best growth while acids (+40) inhibit or retard it. Copious growth on steamed potato cylinders, filling the fluid in the bottom of the tube with yellow slime and converting it into a solid alkaline mass turning brownish with age; nearly complete conversion of potato-starch.

Organism blues litmus milk; it throws down casein slowly, *i.e.*, by a lab ferment; gradual digestion of casein (Smith, Harding); inverts cane-sugar (?); vitality lessened by long cultivation (Harding); slow production of indol in sugar-free peptonized beef-bouillon

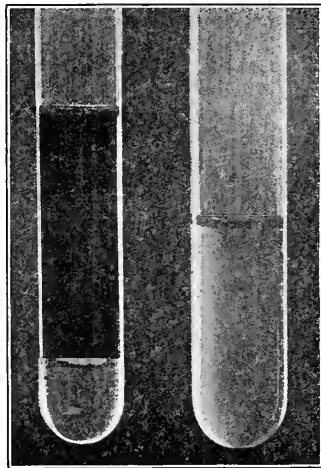


Fig. 130.\*

\*FIG. 130.—Two tubes of bouillon inoculated with *Bacterium campestre*: Left, over chloroform (clear); right, check (clouded). Each tube was inoculated on Feb. 12 with a 2 mm. loop of clouded broth. Photographed Feb. 17.

or peptonized Uschinsky's solution; strictly aerobic so far as is known; occasional formation of hydrogen sulphide; partial reduction of litmus in cabbage-broth cultures; slight production of acid on some vegetable media and with various sugars; scanty growth in Fermi's solution, Uschinsky's solution and sometimes in Cohn's solution (see Negative); feeble growth in vacuo; killed in agar plates by direct sunlight (30 minutes or less); produces a brown pigment soluble in water and a yellow pigment, a lipochrome, insoluble in water but extracted by alcohols, acetone, etc.; minimum temperature for growth is about 5° C., optimum temperature about 30° C., maximum temperature about 38° C to 39° C. Thermal death-point is about 51° C. Tolerates sodium hydrate in peptonized beef-bouillon to -40, and plant acids to +30 or +40 (?). Young cultures stain readily with various basic anilin dyes; sections of tissues stain satisfactorily with Ziehl's carbol-fuchsin, with nigrosin and with Heidenhain's or Benda's iron haematoxylin; a nitro-bacterium according to Fischer's classification (Brenner) see Negative.

Most of the organisms in a test-tube culture were destroyed by two or three freezings and thawings, but a few individuals survived ten. Killed in one minute by a 0.5 per cent solution of lysol but was not killed in 15 minutes by a 0.25 per cent solution (Harding). Carbolic acid in 0.625 per cent solution killed in 5 minutes, but not in 2 minutes (Harding). Less sensitive to the presence of sodium chloride than *Bact. phascoli*. Remained alive 4 to 6 months in agar cultures at room-temperatures (Harding). Lives on culture media for a year in the cool-box (Smith) and on cabbage seed for a year (Harding et al.). Able to live in mixed cultures for a considerable time. Group number 211.3332513 (Smith, Harding).

#### NEGATIVE.

No distinct capsule; no spores; no true pellicle formed on neutral peptonized beef-bouillon (Harding); no acid coagulation of milk; occasionally no liquefaction of unfavorable gelatins; no action on lignified tissues; no solvent action on Swedish filter-paper; no reduction of nitrates to nitrites; not a nitrobacterium (Smith); no characteristic odor; no production of gas or growth in the closed end of fermentation tubes in peptone-water or peptonized beef-bouillon with any of the following carbon compounds: grape-sugar, fruit-sugar, cane-sugar, galactose, milk-sugar, maltose, dextrin, mannitol, glycerin; nor in potato-broth, cabbage-broth or cauliflower-broth; no growth in hydrogen, nitrogen or carbon dioxide; often no growth in Cohn's solution; no growth in peptonized beef-broth over chloroform; brown pigment not formed in beef-broth nor in peptone water with grape-sugar. Not pathogenic to rabbits (Harding).

#### TREATMENT.

The treatment of this disease falls principally under the head of restriction and prevention. Seasonal variations undoubtedly play an important part in the development of the disease on lands already infected. Cool, moist lands may be expected to be more subject to it than warm dry ones. Even in the same field the writer has observed the varying quality of the soil to exert a marked influence on the number of water-pore infections, the plants on the dry end of the field being nearly free. In warm autumns accompanied by frequent rains the infections are much more numerous and the disease certainly progresses much more rapidly than in cool, dry seasons. Garman believed the disease to be fundamentally associated with hot, wet weather. Pammel (1893) says that dry weather in September checked the progress of the disease. Russell notes (1898) that the disease varies much in intensity on the same field in different years according to varying weather conditions. An intelligent cabbage-grower of Racine, Wisconsin, thoroughly familiar with the black rot, recently told the writer that he lost by this disease the entire crop from a field of six acres in the rainy season of 1900, not having enough cabbages even for the use of his



family, whereas from the same field in the dry season of 1901 he harvested a reasonably good crop.\* Under drainage might, therefore, be advantageous in wet seasons.

When the cabbage-plant is well along toward maturity before water-pore infection takes place, to wit, in late summer or early autumn, the disease may sometimes be prevented from entering the head by the removal of infected leaves or portions of leaves† (experiments by the writer, and by Russell), but of course it will not answer to rob the plant of all or most of its leaves and expect a crop, nor is it likely that the experiment would prove very successful when applied to a small part only of a very badly diseased field (Geneva Sta. Bull. 232).

Insects which distribute this disease may, of course, be reduced in number by insecticidal treatments.

Care should be taken that diseased plants do not find their way into the manure-heap, barn-yard, or dump-pile and thence back on to the land. Several cases have come to the writer's attention where wholesale infection of fields or parts of fields could be accounted for only in this way. Potter also mentions one. Heavy manuring appears to favor greatly the development of this disease, but on the other hand considerable manure is required for the satisfactory growth of the plants. The most that can be done is to see that the manure itself does not become infected. Diseased plants should be piled with dry brush and burned, or effectually disposed of in some other way.

Losses in winter in storehouses can be wholly or partially avoided by keeping the plants in all parts of the house at a uniform low temperature, *i. e.*, slightly above freezing. Of course there should be a careful inspection of such heads or roots as they are stored, and those showing decayed spots or blackened bundles should be rejected. Most of the winter decay has been observed to take place in the warmest parts of the houses. Such houses should be well ventilated and uniformly cool.

By far the most common method of infection of healthy fields (so far as we yet know) is through the seed-bed. This should be made with the greatest care, preferably on land not previously used for cruciferous plants, and certainly on land which has never been subject to this disease, and with seeds which are not contaminated by the presence of this organism, otherwise a very considerable proportion of the seedlings may carry the disease with them from the seed-bed into the field, which thus becomes permanently infected. The writer observed one case in Wisconsin where about 20 per cent of the seedlings were diseased. The field set from this seed-bed became so badly diseased that it was abandoned by the planter in midsummer. As early as the first week in September, 58 per cent of the plants set from this seed-bed, (which was on land where the disease prevailed the year before) were diseased, many of them badly. This was a field which had not been previously planted to cabbage and consequently one which might otherwise have been expected to yield a healthy crop.

In 1897 Stewart suggested that the disease is disseminated by seedsmen. This inference was based on the fact that he found the disease in plants which had been reserved for seed and were fruiting, although not, I believe, on the seeds themselves. It is not unlikely that the disease may have been introduced into this country on seed from the old world or that it is now being spread from place to place by cabbage-seed, etc., derived from plants grown on land subject to the disease. This, however, is conjecture and must remain so until some one has demonstrated the actual presence of the organism on cabbage-seed, etc., in spring or at least has obtained diseased plants in healthy soil from the use of such suspected seed. The writer has also seen the disease in cabbage-plants set out for seed and coming into fruit. It is also a well-established fact that much of the cabbage-seed now grown in the United States comes from regions much subject to this disease. The final proof must come

\*The crop on such a field well cared for should be worth \$800.

†Whenever possible, portions only should be removed.

from a study of cabbage and other cruciferous seeds in a natural state, as they come from the seedsman, and most easily before they have left his hands, *i. e.*, in the field on maturing plants observed to be diseased.

Since the above paragraph was written Harding and his associates have thrown a flood of light on this subject by actually obtaining *Bacterium campestre* from the surface of cabbage-seed harvested separately from four diseased plants obtained from a field of seed-cabbage on Long Island. This shows that the organism may reach and contaminate the seeds in a certain number of plants reserved for seed, and renders it likely that the entire crop of a given seedsman would be more or less contaminated if some diseased plants came to maturity in his fields and were harvested along with the sound plants, since the dust of the threshing would disseminate the organisms widely. Can the organisms thus disseminated remain alive for a long time on such seed *i. e.*, from autumn until spring? We do not yet know, but the probabilities are in favor of such a belief, since these experimenters have also shown that when pure cultures of this bacterium are placed on cabbage-seed and dried, some of the bacteria (a small proportion it would seem) remain alive for at least 13 months. These discoveries, while not wholly conclusive, render it extremely probable that the disease is very often disseminated on seed, since the persistent vitality which has been demonstrated experimentally in the laboratory using pure cultures is quite likely to occur naturally, at least sometimes, on contaminated seeds offered for sale in the open market. If the organisms remain alive on such seeds over winter in any great number we have a very satisfactory explanation of the wide dissemination of this disease in the United States during the last 15 years.

Harding has now made out so good a case against the seedsmen that these gentlemen should take very special precautions to avoid harvesting seed from infected plants, and from badly infested fields. Such fields should not be used for production of seed-cabbage. The growers, on the other hand, as a matter of ordinary precaution, should disinfect all cruciferous seeds before planting them. This may be done, it is said, by soaking the seeds for 15 minutes in 1:1000 mercuric chloride water, or in full strength formalin diluted with water in the proportion of 1:240.

It seems likely that this short treatment will prove effective only in case the organisms are confined to the surface of the seeds. If they are sometimes in the interior a longer treatment will probably be necessary, and to avoid this (since it might prove disastrous to germination) it would be advisable to screen out and reject all shriveled and inferior seeds before planting, since it is probable that the plump seeds will be less likely to be infected within than the thin or shriveled ones.

In Farmer's Bulletin No. 68 (which may be had on application to the U. S. Department of Agriculture), the curious reader will find various pieces of evidence tending to show spread of the disease from the seed-bed, persistence of the organism in the soil, and infection of land by way of the dung-pile, and by refuse from cabbage storehouses and pits.

To summarize: *Avoid infected seed, soil, and manures; destroy insect carriers of infection; if the plants are attacked, harvest early, and use at once, or store in a very cool house.*

#### PECUNIARY LOSSES.

It is difficult of course to arrive at any very definite conclusions respecting the losses due to this disease. In September 1904, the writer received the following statement concerning black rot from an extensive cabbage-grower near Chicago:

"I send you by mail some small samples which I hope will be sufficient in order for you to determine the trouble. In some cases as high as 40 or 50 per cent of the cabbage is diseased and in one case which I have noted I should think that over 90 per cent of the cabbage had died from this disease. The ground from which these plants came is comparatively new ground and has only been under cultivation for 2 or 3 years, though large quantities of stable manure have been placed upon it."

The black rot in cauliflower caused a loss of \$400 per acre to a planter at Beeville, Texas, in the winter of 1902, according to his statement to me. Diseased plants were received from him, the organism was plated out on agar, subcultures were made on potato, and then it was re-inoculated into cauliflowers, which became diseased with the typical black rot.

Similar statements respecting losses have been received from many other places (see p. 328). Not infrequently in various parts of the country entire fields have been destroyed so that not a single plant was harvested. Russell has published a very interesting figure of such a field. At the present time the disease is destructive to a greater or less extent, according to the season, on Long Island, in Western New York, and in parts of Ohio, Illinois, Michigan, Wisconsin, and Texas. The disease has come into general notice in this country only recently, but the losses from it in the vicinity of Racine, Wisconsin, were estimated for me by good observers 14 years ago at not far from \$150,000. The disease is also said to be so prevalent in parts of New York that extinction of the industry is threatened. The writer estimates the losses on Long Island during the last 12 years at upward of \$250,000. Probably the total loss from this disease in the United States since its first appearance has been not less than \$800,000.

Hecke reports this disease to be common in Austria on a variety of crucifers. The loss on a kohlrabi field in Southern Austria, which furnished the first material for his studies, was very great, in fact almost complete, since it was impossible to use the black veined tissues for preserving. Van Hall's correspondent in North Holland says that the disease has existed for several years, first attacking Utrecht red cabbage but at present attacking all varieties and even cauliflower. The damage is worst on the red cabbage. The disease is so dreaded that many fear the culture of cabbage will have to be given up. Many hectares of cabbage are diseased this year (1900) so that from seven-eighths to nine-tenths of the harvest will be lost.

In the United States cabbages are often stored in large quantities for the spring market, and the black rot frequently continues in such plants, particularly in the warmer parts of the houses usually associated with soft rots.

According to Russell a quarter of the stored crop of cabbage was lost at Racine, Wisconsin, in the winter of 1896-1897 by the development of this disease in the stock. When taken out such cabbage heads are often sound externally although badly rotted within.

#### HISTORY.

Garman (1891-1892) appears to have been the first to comment on this disease. He saw what we may infer to have been this disease in July, 1889, at Lexington, Kentucky, but did not make out its etiology. From the diseased cabbages two organisms were isolated, a non-motile white and a motile yellow. In most of the cabbages the yellow form was the commoner one. "The size and behavior of this [yellow] species leads me to think it a form of *Bacterium termo*." The two species were not described. A quick decay [soft rot] was obtained by transfer of some of the diseased material to the interior of healthy cabbages, but pure culture inoculations were not successful. Further opportunity for studying the disease did not present itself and the writer leaves the subject with the following remark:

"Whether the disease is induced primarily by the attacks of bacteria, or by hot, damp weather, the work thus far done does not show satisfactorily. From the facts in my possession it appears to me probable that neither alone will cause the disease; that it is only during periods of high temperature and excessive rainfall that the organisms are able to invade and break down the tissues of plants."

Pammel (1893-1895) was the first to demonstrate the infectious nature of the disease. He isolated a yellow bacterium from rutabagas and determined it to be the cause of the disease by means of pure culture inoculations.

In 1896-1897, Smith verified Pammel's statements, extended his inoculations to cabbages and other plants, carefully illustrated the disease in color, and obtained additional information respecting cultural characters of the organism. He also discovered that infections take place through the water-pores. This knowledge, published in June, July, August and September 1897, was also summarized in a Farmer's Bulletin issued January 8, 1898, and widely distributed among cabbage-growers. Subsequently (February and March 1898) Russell, and Russell & Harding, went over the whole ground in papers of considerable length, confirming in the main the statements of Pammel and of Smith, and making various additional observations. The same year, after many additional experiments, Smith again published in "Zeitschrift für Pflanzenkrankheiten."

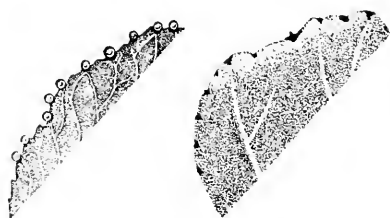
In 1899-1900, Harding demonstrated the disease to be common in Europe. European investigators (van Hall in 1900 and Hecke in 1901-1902) were then moved to study this disease. Hecke in particular did a very thorough piece of work on kohlrabi, adding considerably to our knowledge and at the same time confirming many statements made by Smith, Russell, Harding and others.

In 1901, in his dispute with Fischer, Smith published a series of photomicrographs illustrating this disease. In 1903 he published another series of photomicrographs showing the effect of the black rot on turnips.

In 1903, Potter reported on the occurrence of this disease in England, especially in Swedes.

In 1902-1904, Brenner, a special student of Alfred Fischer at the University of Basel, went over the ground once more at Dr. Fischer's suggestion. He experimented principally with cabbages, although he mentions having observed the disease in numerous other crucifers. He added some new facts and also confirmed many statements previously made by Smith and disputed by Fischer.

In 1903-1905, Stewart & Harding, and Harding, Stewart & Prucha continued their studies, the most important new facts brought out being that the organism does actually



\*Fig. 130a.

occur in autumn on a portion of the cabbage seed grown for market in infected districts, and that when cabbage-seeds are moistened with a culture of *Bacterium campestris* it is able to live on their surface for more than a year, thus rendering it extremely probable that the disease may be disseminated by seedsmen. The manner of threshing cabbage seed, as they point out, is such that the dust from a few infected plants would be likely to contaminate the seeds of many sound plants, if not that of the whole crop.

In 1904-1905, Smith and Swingle showed that the organism could be destroyed in great part by repeated freezings with liquid air and with salt and ice. The few bacteria which survive the freezings are still infectious.

In 1910, Harding summarized his studies on about 45 isolations of this organism in accordance with the requirements of the Descriptive Chart of the Society of American Bacteriologists.

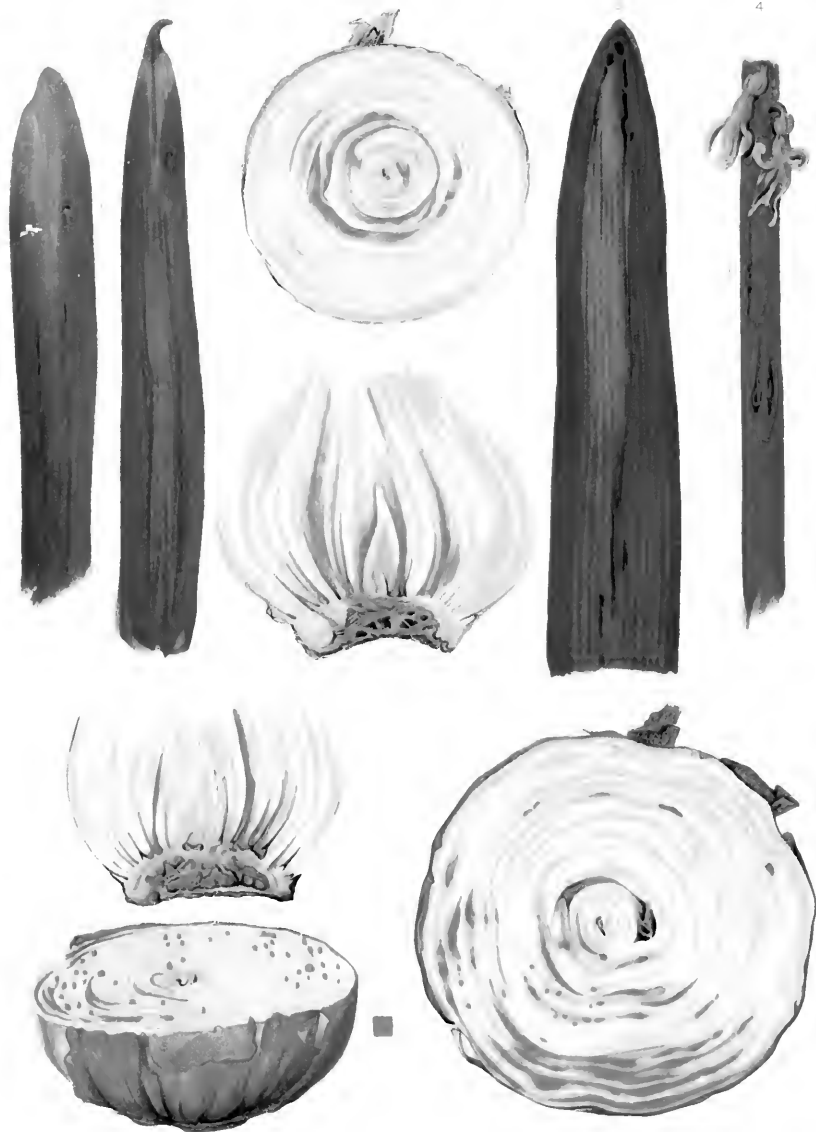
\*FIG. 130a.—Left: Margin of cabbage leaf showing extrusion of fluid from the water-pores. Right: Early stage of bacterial infection on cabbage leaf by way of the water-pores.

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This organism was obtained from cabbage seed taken from plants diseased by black-rot.  
On cabbage seed soaked in water to which *Ps. campestris* was added and then dried and placed in test-tubes some of the bacteria were alive at the end of 10 months. See bull. 251.
1914. HARDING, H. A., STEWART, F. C. and PRUCHA, M. J. Vitality of the cabbage black-rot germ on cabbage seed. *Bull. 251*, New York Agric. Exp. Sta., Geneva, N. Y., Oct. 1904.
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"It was observed that some kinds were more severely injured than others. For example the Jersey Kale was more diseased than any other kale."
1907. KIRK, T. W. Black-rot of cabbage. *Ann. Report*, New Zealand Department of Agriculture, 1907, vol. XV, p. 157.  
Kirk reports having seen black-rot of cabbage due to *Bacterium campestris* in two different years in New Zealand.
1908. EDWARDS, S. F. Cabbage resistant to black-rot. In 33d Annual Report, Ontario Agricultural College and Experimental Farm for the year 1907. Toronto, 1908, p. 134.
1908. JANCZEWSKI, A. *Pseudomonas campestris* (Bacteriosis of cabbage). Jahresbericht für 1907 (Russian), St. Petersburg, 1908, p. 71.
1908. FAWCETT, H. S. Cabbage Disease. Black Rot (*Pseudomonas campestris* (Pammel) Erw. Smith). *Florida Agric. Exp. Sta., Ann. Report* for 1908, pp. LXXV-LXXX. Also Press Bulletin, 101.  
Reports "serious loss to cabbage, cauliflower, and ruta-baga crops in the State for several years." At Sutherland in February, 1908, "an examination of the fields showed that the black rot was prevalent throughout the section, destroying from 25 to 75 per cent of the crops. Cultures made from diseased plants revealed the presence of yellow bacteria (*Pseudomonas campestris*) in specimens of the three plants named."
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Describes cultures of *Ps. campestris* made as test of classification card. Soc. American Bact.





# YELLOW DISEASE OF HYACINTHS

(1) Plate 1, fig. 24, (2) Plate 2, (3) Hyacinth, 49x magnification, at x. Pansied  
 (4) Hyacinth, 20x magnification, at x. (5) Hyacinth, 20x magnification, at x.  
 (6) Hyacinth, 20x magnification, at x. (7) Hyacinth, 20x magnification, at x.  
 (8) Hyacinth, 20x magnification, at x. (9) Hyacinth, 20x magnification, at x.  
 (10) Hyacinth, 20x magnification, at x. (11) Hyacinth, 20x magnification, at x.  
 (12) Hyacinth, 20x magnification, at x. (13) Hyacinth, 20x magnification, at x.  
 (14) Hyacinth, 20x magnification, at x. (15) Hyacinth, 20x magnification, at x.  
 (16) Hyacinth, 20x magnification, at x. (17) Hyacinth, 20x magnification, at x.  
 (18) Hyacinth, 20x magnification, at x. (19) Hyacinth, 20x magnification, at x.  
 (20) Hyacinth, 20x magnification, at x.



## YELLOW DISEASE OF HYACINTHS.

(Synonyms: Wakker's Bacterial Disease; Yellow Slime—Dutch *Geele Snot*.)

### DEFINITION.

This is a specific communicable disease of the common hyacinth, the most characteristic sign of which is the appearance in the bulbs of a bright yellow bacterial slime (pl. 19). On cross-sections of the bulb the disease appears in the earlier stages as small yellow dots, and on the longitudinal section as long, narrow, yellow stripes, corresponding to the location of the vascular bundles, which are the first parts to be conspicuously infected. In later stages the parenchyma is involved, the bulbs are badly decayed, and there are then various secondary infections (see plate 20, figs. 7, 11). Other signs are dwarfing and one-sided growth of the foliage, and water-soaked or brown stripes on the leaves.

### HOST-PLANTS.

This disease is known to occur only in the common Dutch hyacinth (*Hyacinthus orientalis*). It has been successfully inoculated into this species. The leaves of the inoculated plants showed the characteristic stripes and at the end of some months unmistakable signs also developed in the interior of many of the bulbs. It has also been inoculated by the writer into the leaves of *Hyacinthus albulus*, *Allium cepa*, and *Amaryllis atamasco*, but only with slight local results; in no case did the bulbs of these plants become affected. No results were obtained from inoculation into the leaves of cabbage.

### GEOGRAPHICAL DISTRIBUTION.

So far as known the disease occurs only in the Netherlands, where the hyacinth is grown in vast gardens for export to all parts of the world.

### SIGNS OF THE DISEASE.

The first sign in an inoculated leaf consists of stripes having a water-soaked appearance (plate 19, figs. 1 to 3). These are soon followed by the yellowing, browning and death of the tissue first attacked and by the appearance of water-soaked spots or stripes farther down, which in turn die and dry out. Sometimes the killed tissue becomes more or less transparent except the veins, which are feebly browned. In natural infections, these stripes usually begin toward the apex of the leaf. They extend downward rather slowly, but much more rapidly in this direction than sidewise. The result is that often the leaf will come to have a central dead stripe extending nearly or quite its whole length, while the margins of the leaf are still green and healthy in appearance. Sometimes the bulbs are infected from the flower-stalk. The signs in the scape lower down may or may not be external. When externally visible there is a water-soaked appearance (plate 19, fig. 4 and plate 20, fig. 5), followed by browning and shriveling. The signs in the bulb are so striking as to be unmistakable. In early stages of bulb-infection the disease is confined quite strictly to the vascular bundles, from one to fifty or more of these being yellow and full of bacterial slime, in a white and otherwise healthy tissue (fig. 131, and plate 19, fig. 8). When the infection occurs through leaves, the scales which bear these particular leaves are the first part of the bulb to show the yellow slime, and naturally this appears first at the top of the scale in the vascular bundles. A little later the bacterial slime from these particular scales grows down into the solid base of the bulb (the plateau) where many of the numerous

anastomosing vascular bundles become yellowed, and where we often find a considerable area of the intervening parenchyma yellow and soggy (plate 19, figs. 6, 7). Subsequently the yellow slime extends upward into the bundles of other scales and sidewise slowly into the parenchyma, until finally the bulb is destroyed. In the later stages of the disease small pockets occur in the bulb-scales (plate 20, figs. 8, 9) and other bacteria frequently enter and help to complete the destruction of the bulbs but their presence is not essential.



Fig. 131.\*

the other (plate 19, fig. 9), and this may result in a curved growth of the foliage which bends over toward the diseased side.

#### ETIOLOGY.

The cause of this disease is *Bacterium hyacinthi* Wakker; a bright-yellow, medium-sized rod with rounded ends, motile by means of one polar flagellum, and multiplying by fission. It is this organism which causes the yellow color in the bundles of the diseased bulbs. The yellow slime in the bulbs is made up entirely of a homogeneous-looking bacterial growth which in early stages ordinarily yields pure cultures of this organism when cultivated out with any degree of care, but which is sometimes mixed with other organisms, especially in advanced stages of the disease. Wakker, who first studied this disease critically, obtained at different times a number of good cases as the result of wounds made in the leaves, scapes and bulbs, but inasmuch as most of his successful inoculations were what the writer has designated *direct* infections, i.e., the inoculation of raw material, there has been a tendency on the part of various writers to discount his results, and to confuse the general reader by speculations not based on any experimental data. Dr. Wakker's statements are, however, in the main, trustworthy, since the writer has obtained numerous successful confirmatory inoculations from pure cultures of this yellow organism (for figures consult Bulletin 26).

\*FIG. 131. Early stage in the destruction of a hyacinth bulb by *Bact. hyacinthi*. Cross-section of bulb enlarged to show diseased vascular bundles in 3 scales. These 6 bundles were bright yellow. A seventh bundle, which does not show plainly in the cut, was also diseased. Several of the dark spots are negligible, being shadows due to slight openings between the scales. Plant inoculated Feb. 16, 1897, on upper part of scape. Photographed June 23, 1897. Circa x 3.





The period of incubation in the writer's experiments varied from 3 to 30 days depending on the amount of infectious material employed and on the susceptibility of the variety. All of his inoculations were through the leaves and floral organs, and always at a considerable distance from the bulb. In all cases pure cultures were used for the inoculations which were made in various ways, *viz.*, by needle-punctures, by hypodermic injection, by placing drops of infectious fluid in the flowers, and by submerging the tips of leaves in fluid containing the living organism. The last method led to no very conclusive results but since the writer's experiments were not numerous and yet gave some indications of ultimate success, they should be repeated with distinctly negative results before we are warranted in asserting that it is impossible to communicate the disease by way of the stomata. The other three methods were each successful.

A great many leaf-infections were obtained and forty of the inoculated plants also showed the characteristic signs in the bulbs at the end of 2 to 4 months. From the interior of the bulbs which became diseased in this manner this same organism was re-isolated on several different occasions and grown in pure cultures which again produced the typical disease when re-inoculated. All the several hundred control plants maintained by the writer continued free from this disease. There remains, therefore, no good ground for doubting the general correctness of the statements advanced by Wakker as to the cause of this disease. It is not only a genuine bacterial disease, but one of the most peculiar and interesting vascular diseases known to the writer.

The natural methods of infection (except from mother-bulbs to daughter-bulbs) are not well understood. The disease is readily induced through wounds and it is likely that the knife of the gardener is responsible for a portion of the infections. Inasmuch, however, as in many of the plants the signs are said to begin on the leaves at a considerable distance from the ground some other explanation must be sought, at least for a portion of the infections. On several occasions the writer succeeded in producing the disease in the bulbs by putting drops of infectious fluid into the flowers (fig. 132). It is possible, therefore, that the disease may be disseminated both by leaf-eating and by nectar-sipping insects. Signs have not been observed in the roots.

According to Wakker, wet weather greatly favors the progress of the disease, while sunshine and dry weather are unfavorable to it. This is true, also, of many other bacterial diseases, *c. g.*, tobacco-wilt and pear-blight.

#### VARIETAL RESISTANCE.

Twenty-five years ago it was common observation, according to Dr. Wakker, that some varieties were very little subject to this disease in fields where other varieties were badly attacked. He seems to have had no doubt about the "predisposition" of certain

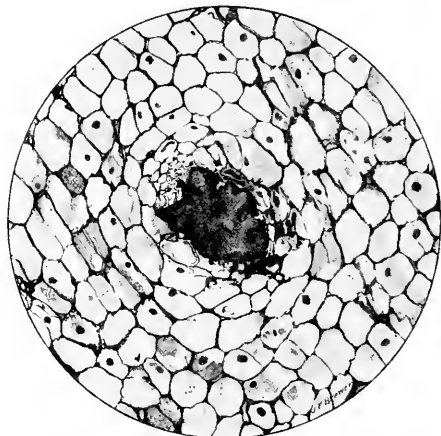


Fig. 132.\*

\*FIG. 132.—Cross-section of base of hyacinth bulb showing cavity in the bundle due to *Bacterium hyacinthi*. Plant No. 67 inoculated through the flowers. Slide 502 A-A9. Drawn with Zeiss 16 mm. and 12 ocular.

varieties to attack. He obtained lists of sensitive and resistant varieties from seven growers. A study of these lists showed a very general agreement upon the following varieties as very susceptible:

Snowball, *single white*;  
Grand Lilas, *single blue*;  
La Tour d'Auvergne, *double white*;  
Duchess of Richmond, *single red*;  
L'Ornement de la Nature, *single red*;  
Oran dates, *single blue*;  
Mirandolina, *single white*;

De Candolle, *single blue*;  
Princess héréditaire des Pays-Bas, *single white*;  
Grand Vainquer, *single white*;  
Mimosa, *single blue*;  
Alba Maxima, *single white*;  
John Bright, *single blue*;

The following varieties were regarded by the same growers as resistant, or only slightly susceptible:

Pieneman, *single blue*;  
Norma, *single red*;  
Pucelle d'Orleans, *single white*;  
Voltaire, *single white*;

Argus, *single blue*;  
Bleu mourant, *single blue*;  
Willem I, *single blue*;  
Maria Catherina (Robt. Steiger), *single red*.

These two lists are copied from Wakker's French paper published in Archives néerlandaises.

In 1906 two of the leading hyacinth growers of Holland, men who have been in the business many years, were good enough to go through their catalogues and mark off for me, independently, varieties "much subject" to *Bacterium hyacinthi*, and varieties "very resistant," leaving those varieties unmarked which are intermediate in resistance, or about which there might be a difference of opinion or a lack of exact knowledge. In this way I obtained information concerning about 300 varieties. I have carefully compared the two lists and find them flatly contradictory only in the case of three varieties—single red Pélissier, single rose Maria Cornelia, and single white L'Innoceuce. There are, however, numerous discrepancies, many varieties being marked + or o by one grower and left unmarked by the other. This might mean either entire lack of knowledge, or difference of opinion. In case it were disagreement we might assume either difference of behavior on the part of particular varieties in the hands of different growers, something not improbable, or that one man is a closer observer than the other. Of course if a variety is much subject to the disease in one field and resistant to it in another, the observed resistance might be entirely a matter of accident and not due to any strongly inherent peculiarity, of which one might take advantage in cross-breeding or selection. Varieties to the number of 130 were marked + or o by one or other of the two growers, 73 as much subject to the disease, and 57 as very resistant. One grower reported 59 varieties "much subject" and 43 "very resistant," the other reported 22 varieties "much subject" and 32 "very resistant." The agreements are of greater interest. There are 26 of these, a number apparently too large to be purely accidental. In addition it should be mentioned that a number of the older varieties, marked + in one catalogue, are not included in the lists of the other dealer, but must at one time have been grown by him, and may have been discarded on account of disease. The writer started inquiries to determine this point and found this supposition correct. Including these, also, the number of agreements is 35.

The writer subsequently received a catalogue from a third large grower, a person well-known in the trade for thirty years or more. This man marked 61 varieties "much subject" and 13 varieties "very resistant." The varieties concerning which this third grower is in agreement with the other two growers are marked with an asterisk in the following table. Respecting the other varieties in this table he makes no statement. He is in contradiction with one or other of the two growers, never with both, respecting the susceptibility of three varieties: Clio, *single light blue*; Obelisk, *single yellow*; and Czar Nicholas, *double rose*.

He reported on 41 varieties not mentioned by the other growers, 5 being marked as resistant.

The varieties concerning which the two growers agree are included in the following lists: The starred varieties represent the agreements subsequently received from a third large grower.

Table Showing Sensitive and Resistant Varieties of Hyacinth.

Hyacinths much subject to the yellow disease.	Hyacinths very resistant to the yellow disease.
Charles Dickens, <i>single rose</i>	*Robert Steiger, <i>single red</i>
*La Grandess, <i>single white</i>	Baron van Tuyll, <i>single rose</i>
*La Neige, <i>single white</i>	Moreno, <i>single rose</i>
Captain Boyton, <i>single light blue</i>	Baroness van Tuyll, <i>single white</i>
*Czar Peter, <i>single light blue</i>	*Grandeur à Merveille, <i>single bluish white</i>
*Grand Lilas, <i>single light blue</i>	La Franchise, <i>single bluish white</i>
*Lord Derby, <i>single light blue</i>	*Grand Maître, <i>single light blue</i>
*Hermann, <i>single yellow</i>	*La Peyrouse, <i>single light blue</i>
*La Tour D'Auvergne, <i>double white</i>	Regulus, <i>single light blue</i>
To this list the following should be added	
as a result of the additional inquiry respecting discarded sorts already mentioned	
La Précoce, <i>single white</i>	*Marie, <i>single dark blue</i>
Marie Stuart, <i>single white</i>	King of the Yellows, <i>single yellow</i>
Miss Nightingale, <i>double white</i>	Yellow Hammer, <i>single yellow</i>
*Mont Blanc, <i>single white</i>	Princess Royal, <i>double red</i>
*Orandates, <i>single light blue</i>	Boquet Royal, <i>double rose</i>
Prince de Taillerand, <i>single light blue</i>	La Virginité, <i>double bluish white</i>
*King of the Blacks, <i>single blue black</i>	Crown Prince of Sweden, <i>double dark blue</i>
*La Citronière, <i>single yellow</i>	
Mimerva, <i>double yellow</i>	

†Found also accidentally by the writer in 1897 to be specially sensitive to pure-culture inoculations of *Bact. hyacinthi*.

For comparison with the above table I have compiled the following list from Wakker's Dutch report for 1885, the same being the opinion of six hyacinth growers of that time respecting susceptibility to this disease of 34 of the 35 varieties mentioned in the preceding list. When the sum is less than six the other growers made no report.

Variety.	Number of growers reporting the variety as:		
	Very susceptible	Intermediate.	Not susceptible.
Charles Dickens, <i>single rose</i> . . . . .	—	—	3
La Grandess, <i>single white</i> . . . . .	—	1	3
La Neige, <i>single white</i> . . . . .	3	1	—
Captain Boyton, <i>single light blue</i> . . . . .	—	—	—
Czar Peter, <i>single light blue</i> . . . . .	—	3	3
Grand Lilas, <i>single light blue</i> . . . . .	6	—	—
Lord Derby, <i>single light blue</i> . . . . .	3	1	—
Hermann, <i>single yellow</i> . . . . .	—	—	4
La Tour d'Auvergne, <i>double white</i> . . . . .	6	—	—
La Précoce, <i>single white</i> . . . . .	—	—	—
Marie Stuart, <i>single white</i> . . . . .	—	—	1
Miss Nightingale, <i>single white</i> . . . . .	3	—	—
Mont Blanc, <i>single white</i> . . . . .	—	—	6
Orandates, <i>single light blue</i> . . . . .	5	—	—
Prince de Taillerand, <i>single light blue</i> . . . . .	2	—	—
King of the Blacks, <i>single blue black</i> . . . . .	—	—	—
La Citronière, <i>single yellow</i> . . . . .	1	1	—
Mimerva, <i>double yellow</i> . . . . .	—	—	—
Robert Steiger, <i>single red</i> . . . . .	—	1	5
Baron van Tuyll, <i>single rose</i> . . . . .	—	—	3
Moreno, <i>single rose</i> . . . . .	—	—	1
Baroness van Tuyll, <i>single white</i> . . . . .	—	—	—
Grandeur à Merveille, <i>single bluish white</i> . . . . .	—	—	5
Grand Maître, <i>single light blue</i> . . . . .	—	—	2
La Peyrouse, <i>single light blue</i> . . . . .	—	—	1
Regulus, <i>single light blue</i> . . . . .	—	2	2
King of the Blues, <i>single dark blue</i> . . . . .	—	2	2
Marie, <i>single dark blue</i> . . . . .	—	—	6
King of the Yellows, <i>single yellow</i> . . . . .	—	—	—
Yellow Hammer, <i>single yellow</i> . . . . .	—	—	—
Princess Royal, <i>double red</i> . . . . .	—	—	1
Boquet Royal, <i>double rose</i> . . . . .	—	—	1
La Virginité, <i>double bluish white</i> . . . . .	—	—	3
Crown Prince of Sweden, <i>double dark blue</i> . . . . .	1	1	1

From a comparison of these two tables it appears that none of the varieties regarded as very susceptible twenty five years ago have ceased to be susceptible, but rather, that some of them have been discarded by certain growers on this account. On the contrary, some of the varieties then regarded as very resistant continue to be very resistant, *e. g.*, Robert Steiger, *single red*; Baron van Tuyll, *single rose*; Grandeur à Merveille, *single bluish white*; Marie, *single dark blue*. It also appears that some varieties then regarded as resistant are now classed as very susceptible, *e. g.*, Charles Dickens, *single rose*; La Grandess, *single white*; Czar Peter, *single light blue*; Hermann, *single yellow*, and Mont Blanc, *single white*.

Respecting these latter cases, we may assume (1) that they have changed in this particular during the last two decades, or (2) that they were susceptible from the start, but had not at that time been subjected to rigorous tests calculated to bring to light their inherent susceptibility.

For the sake of those who may desire to see all the discrepancies, I append also my entire list, premising that o means *very resistant*; + *much subject*; and § *intermediate or no report*. To find the varieties not marked by these growers the reader has only to consult any good catalogue of Dutch bulbs.

Table giving complete list of varieties mentioned by one or more of the three Dutch hyacinth growers in 1906, as resistant or susceptible to the yellow disease:

Single red:	Jenny Lind § +	King of the Blues o o o
Amy o § §	La belle Blanchisseuse o § §	La nuit § § +
Etna § § o	La Grandesse + + +	Leopold II o § §
Garibaldi + § §	La neige + + +	Lord Mayo § +
Gertrude § o o	La précoce + § §	Marie o o o
Homerus § o o	L'Innocence o + §	Baron von Humboldt + § §
Incomparable + § §	Madam van der Hoop § § +	General Havlock + § +
King of the Reds + § §	Mary Stuart + § §	King of the Blacks + § +
Linnaeus + § §	Mina § § +	Masterpiece + § +
Pélessier o + §	Mont Blanc + § +	Mimosa § + +
Prima donna + § §	Paix de l'Europe § § o	Pasteur + § §
Queen Mary + § §	Mr. Plimsoll § o §	Sir Henry Barkley + § +
Robert Steiger o o o	Queen of England + § §	Uncle Tom § § +
Roi des Belges § § o	Snowball + § +	William I + § +
Von Schiller + § §	White Bird o § §	William III § § +
Vuurbaak + § §	Grandeur à Merveille o o o	
	La Franchise o o §	
	Lord Grey + § §	Single violet, lilac, and mauve:
	Mammoth o § §	Distinction o § §
	Voltaire o § §	Lady Stanhope § § +
Single rose:		Laura § § +
Baron v. Tuyll o o §		L'Honneur d'Overveen + § §
Beauty of Waltham + § §		Lilas énorme § § +
Carlyle + § §		L'Unique § § +
Charles Dickens + + §		Mauve Queen § o §
Dr. Livingstone + § §	Single light blue:	Sir Edwin Landseer § § +
Maria Cornelia + o §	Blondin § § o	Sir William Mansfield § o §
Gertrude § § o	Captain Boyton + + §	
Gigantea o § o	Clio § o +	
La joyeuse + § §	Czar Peter + + +	
Lady Derby o § §	Enchantress § § +	
Moreno o o §	Grand Lilas + + +	
Norma o § §	Grand Maître o o o	Single yellow and orange:
Princess Hélène § § +	La Peyrouse o o o	Anna Carolina + § §
Rosea maxima o § §	La Precieuse + § §	Bird of Paradise + § +
Rosine § o §	Leonidas + + +	City of Haarlem § § +
Sophia Charlotte § § +	Lord Beaconsfield § § +	Duc de Malakoff § § +
Sultan's Favorite § § +	Lord Derby + + +	Hermann + + +
Windhorst § § +	Lord Palmerston + + +	Ida + § +
	Orandates + § +	King of Holland § § +
	Pieneman § o §	King of the Yellows o o §
	Prince de Tailleraud + § §	La Citronnière + § +
Single white:	Princess Mary of Cambridge + § §	La Grande Jaune § § +
Alba maxima § § +	Queen of the Blues § § +	L'Or d'Australie § § +
Alba superbissima § § +	Regulus o o §	Marchioness of Lorne § § +
Albertine § + §	Schotel + § +	Obélisque o § +
Angenis Christiana § o §	Turquoise § o §	Yellow Hammer o o §
Baroness van Tuyll o o §		
Blanchard § § +		
British Queen § § +	Single dark blue:	
Crown Princess + § +	Argus § § +	Double red:
Grand Vainqueur § § +	Bleu mourant § o §	Kastanjebloem o § §
Grande Vedette + § §	Baron van Tuyll § o §	Noble par mérite o § §
		Princesse Louise + § +
		Princesse Royale o o §



## Double rose:

*Boquet royal* o o §  
*Boquet tendre* § § +  
*Czar Nicholas* o § +  
*Dagmar* § § +  
*Frederick the Great* § § +  
*Globosa* § § +  
*Grootvorst* o § §  
*Leo* + § §  
*Le Grand Concurrent* o § §  
*Princesse Alexandra* § o §  
*Princesse Louise* § § +  
*Venus de Medici* + § §

## Double white:

*Boquet Royal* § § §  
*Flevo* § o o  
*Florence Nightingale* + § +  
*Grand Vainqueur* + § +  
*Jenny Lind* § § +  
*La Grande Duchesse* § § +  
*La Grandesse* + § +  
*La Tour D'Auvergne* + + +  
*Princesse Metternich* § § +  
*Isabella* o § §



Fig. 133.\*

## Double white—continued:

*La Virginité* o o §  
*Prince of Waterloo* + § §  
*Triumph Blondine* o § §

## Double light blue:

*Madame Monmouth* + § §  
*Rembrandt* o § §  
*Van Speyck* + § §

## Double dark blue:

*Crown Prince of Sweden* o o §  
*Laurens Koster* + § §  
*Lord Raglan* o § §  
*Prince of Saxe Weimar* o § §

## Double yellow and orange:

*Boquet d'Orange* + § §  
*Minerva* + § §  
*Sir Rowland Hill* § § §  
*Sovereign* o § + (by letter §)  
*Sunflower* o § §

## Double violet:

*La Victoire* + § §

From the third grower I received the following letter and supplementary list of varieties:

I have much pleasure in handing you a catalogue in which, according to your suggestion, are marked the hyacinths which are much subject to the yellow disease and such which are very resistant. It must, however, be understood that similar statements have only relative value. Many varieties which used to be among the best trade-sorts a few years ago, have now entirely disappeared in consequence of the yellow disease and it may be expected that the list of varieties after ten years will be very much reduced.

The following list of hyacinth varieties shows those which have entirely or nearly disappeared in consequence of the yellow disease:

## Single red and rose:

*Émilius*  
*Ornement de la Nature*  
*Mrs. Beecher Stowe*  
*Unica spectabilis*  
*Duchesse de Richmond*  
*Mars*  
*Lord Grey*  
*Lord Percy*

## Single white:

*Madame de Staël*  
*Queen Victoria*  
*Miss Nightingale*

## Single blue:

*Orondates*  
*Émilius*  
*Prinz Albert von Preussen*  
*Nimrod*  
*Siam*  
*Grand Vainqueur*  
*Argus*  
*De Candolle*  
*Porseleinen Scepter*  
*Priestley*  
*Ferruck Khan*  
*von Schiller*  
*Clio*

## Single blue—continued:

*King of the Blacks*  
*The Sultan*

## Single yellow:

*La Pluie d'Or*  
*Overwinnaar*  
*La Citronière*

## Single violet:

*Haydn*  
*Jesko*  
*Sir Henry Havelock*

## Double red:

*Regina Victoria*

## Double white:

*Anna Maria*  
*van Hoboken*  
*Mont St. Bernard*  
*Emperor of the double Whites*

## Double blue:

*Louis Philippe*  
*Lord Raglan*  
*Frans Hals*  
*Duke of Norfolk*

## Double yellow:

*Jaune suprême*

\*FIG. 133.—Detail from a cavity made by *Bact. hyacinthi* in a bulb-scale of hyacinth showing separated and crushed cells lying in a mass of bacteria—cavity like that shown in fig. 132, but larger. Slide 507 B 6, third section from right. Material collected by the writer at Haarlem in 1906. Gram's stain modified by substitution of amylc alcohol for ethyl alcohol. Starch grains on bottom at right.

The *new* varieties are also cataloged by one of the growers, and of these 7 are marked as much subject and 6 as very resistant, while 31 do not fall into either class.

Wakker states that most of the leaf-infections are usually observed in the fields in the month of May, but thinks that infection must take place at least a month earlier. The writer's experiments have led him to the same conclusion. Undoubtedly the bulk of the field infections occurs during blooming time, when insects would be visiting the blossoms freely.

The downward movement of the disease in the leaves is very slow (Wakker, Smith).

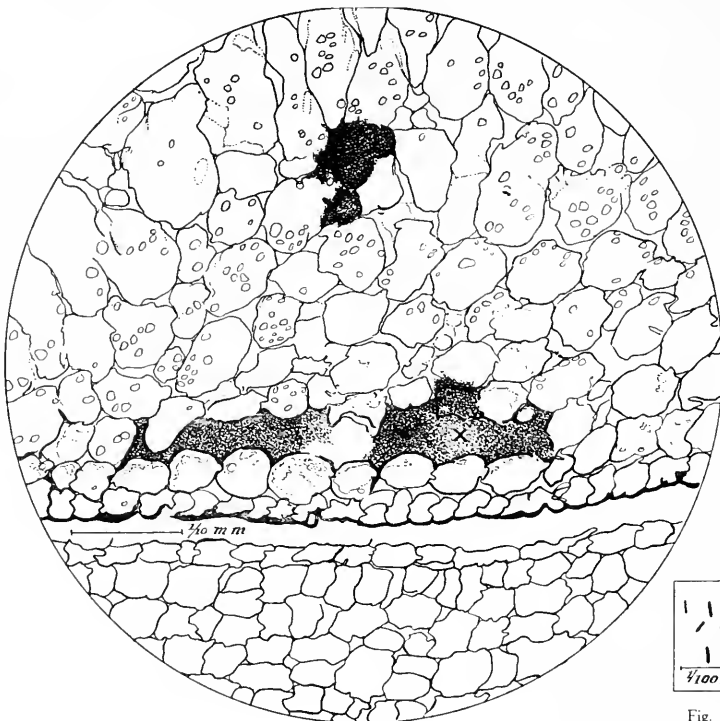


Fig. 134.\*



Fig. 135\*.

### MORBID ANATOMY.

There are no hyperplasias in connection with this disease. It is primarily, and to a considerable extent during its whole progress, a disease of the vascular bundles. The reasons for this the writer has attempted to set forth in his papers on this organism (see Literature). They are noted briefly under the next head. The xylem portion of the bundle is the first part to be attacked, especially the spiral vessels which are soon filled entirely by the rapid multiplication of the organism (plate 20, fig. 6). This is what causes the bright

\*FIG. 134. Cross-section of base of a hyacinth bulb, showing cavities in parenchyma due to *Bact. hyacinthi*. Upper part of drawing is extreme base of a bulb scale; lower is part of plateau. Starch grains are represented in outline only. Slide 502 A. A3, from plant No. 67 inoculated in the flowers (see Bull. 26, p. 30).

\*FIG. 135.—*Bact. hyacinthi*: A detail from fig. 134 at  $\times$ .

yellow color so conspicuous in the attacked bundles. Often the xylem is the only part of the bundle attacked. When the vessels become thus occluded the walls give way, probably by solution, and the bacteria flood out into the surrounding parenchyma, which, however, is quite resistant. In the end, cavities are formed in the parenchyma surrounding the bundles, but the progress of the disease in this tissue is extremely slow. These cavities are filled with remnants of the vessels and cells of the host-plant and by enormous numbers of the yellow bacteria. In the formation of cavities in the parenchyma the intercellular spaces are first occupied (figs. 133, 134, 135). In all of the diseased bulb-scales examined by the writer prior to 1906 the bulk of the tissues was still sound and the organisms were either confined to the bundles, or had made comparatively small cavities around the same. In several hundred bulbs examined in Holland, in August, 1906, the disease had made more extensive inroads (fig. 136) and large areas, especially of the inner face of the diseased scales, were yellow and gummy (plate 20, figs. 8, 9, 10). The disease seems to progress a little more rapidly in the base of the bulb, where there is a net-work of vessels in rather close connection. Here also cavities are formed in the tissues. A small portion of the base of a bulb in an early stage of infection is shown in fig. 132. In the end the whole plateau becomes yellow and gummy and the surface is ruptured, letting in various molds and bacteria. The writer has not attempted to cut many sections of diseased leaves, but Wakker did so carefully, after fixing in absolute alcohol, and showed that here also the downward movement of the organism is through the spiral vessels of the xylem. The few I have cut and examined confirm Wakker's view (see figs. 137, 138). In the end, the whole plant is destroyed, but, so far as the writer has observed, when the disease is uncomplicated, there is never anything resembling a soft white rot, such as that described by Heinz. In none of the many bulbs examined by the writer in 1897-1901 had the disease progressed far enough for the organism to break out of individual scales and pass sideways into the open spaces between the scales, but this phenomenon was observed in Holland in 1906.

Although the action on the cell-walls is slow, there can be little doubt I think that in the end the cell-wall proper as well as the middle lamella is dissolved and disappears. I have not established this fact, however, beyond dispute.

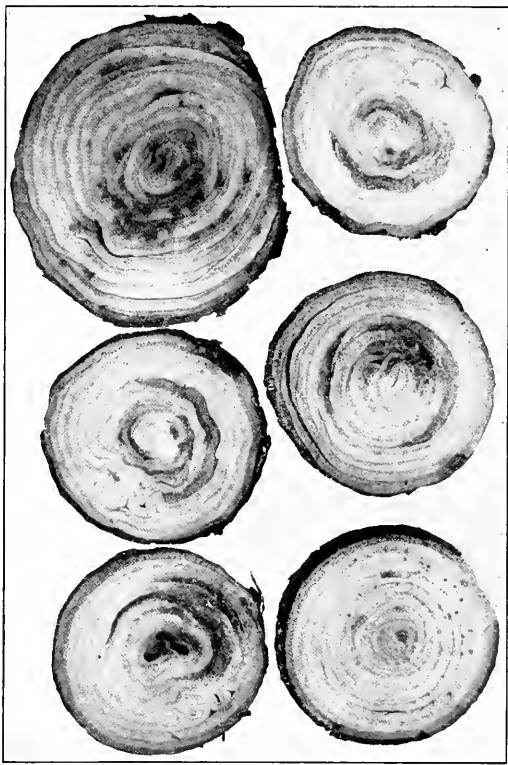


Fig. 136.\*

\*Fig. 136.—Cross-section of 6 hyacinth bulbs from a field near Haarlem, showing advanced stages of the yellow disease due to *Bact. hyacinthi*. Photographed by the writer in the summer of 1906.

## THE PARASITE.

*Bacterium hyacinthi*\* Wakker is readily isolated. In the plant and on agar and in beef-broth, etc., it is a short rod, single or in pairs, or more rarely in fours joined end to end (figs. 139, 140). Rarely short chains have been observed, e. g., on agar. It measures under these circumstances  $0.4$  to  $0.6 \mu \times 0.8$  to  $2 \mu$ , but like many other organisms, it is longer or shorter, thicker or thinner, according to age, culture-medium, and kind of stain used. It is generally slenderer than *Bact. campestre* or *Bact. phaseoli*. The following are some measurements:

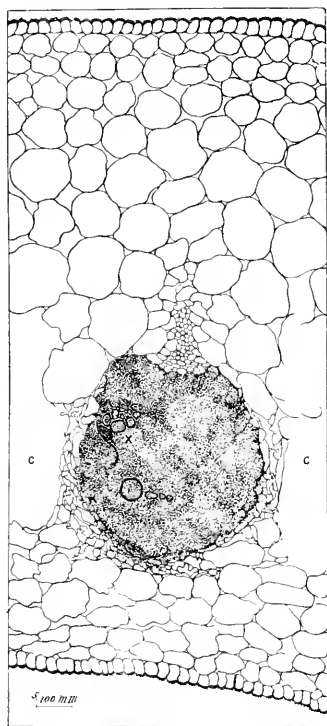


Fig. 137.†

(1) February 5, 1898. Slime from a daughter-bulb stained 5 minutes in a saturated solution of basic fuchsin (very weak stain). Rods short,  $0.5$  to  $1.0 \times 0.4$  to  $0.5 \mu$ . Two minutes in saturated watery solution of Gentian violet gave a deeper stain but not deep enough.

(2) February 7, 1898. Alkaline beef-broth, No. 1, January 29, 1898, stained 10 minutes in saturated watery solution of basic fuchsin.

$2.0 \times 0.4 \mu$	Single rods.	
$1.4 \times 0.4 \mu$		
$1.0 \times 0.4 \mu$		
$3.6 \times 0.4 \mu$		two rods joined end to end.
$3.2 \times 0.4 \mu$		two rods joined end to end.

Widest rods seen  $0.6 \mu$ .

(3) July 31, 1898. Slide of March 10 from very dilute beef-broth 3 days old, Moore's flagella stain. Size  $1$  to  $2 \times 0.5$  to  $0.7 \mu$ . Flagella 3 times length of rods.

(4) August 3, 1898. Slide of March 17, 1897, agar stock 207, Fischer's flagella stain:

$2 \times 0.8 \mu$	$2$ to $3 \times 1.0 \mu$ .
$2 \times 1.0 \mu$	$2.5 \times 0.8$ to $1.0 \mu$ several.

(5) August 3, 1898. Slide of June 23, 1897, made from the interior of a bulb (yellow slime). Plant inoculated on leaf February 16, 1897. Stain, basic fuchsin in water.

$1.0 \times 0.5 \mu$  two;  $1.5 \times 0.5 \mu$ ;  $1.2 \times 0.5 \mu$ ; Most  $1$  to  $1.2 \times 0.5 \mu$ ;  
Extremes,  $0.9$  to  $1.5 \times 0.5 \mu$ .

Pseudozoogloæ are common. No spores have been discovered by the writer, and the spores described by Wakker probably belonged to some other organism.† This is the more likely because the cultures in which they developed abundantly were made directly from the bulb, i. e., not from colonies, and were kept at a temperature slightly above the maximum for the growth of this organism, as determined by the writer. Making cultures from bulb scales in the same way as Dr. Wakker, the writer has twice obtained mixed growths from what looked like an unmixed source; the yellow organism being contaminated once by a green fluorescent organism and once by a white, gas-forming species. In the plant and in the common culture media chains and filaments do not occur, or are rare, but old cultures on media rich in sugar, e. g., streaks on dextrose-agar or saccharose-agar, often

\*Synonyms: *Bacillus hyacinthi* (Wakker) Trevisan; *Pseudomonas hyacinthi* (Wakker) EFS.

†The endospores observed by Wakker were blue-shining, strongly refractive bodies, germinating equatorially. They were cylindric with rounded ends, measuring  $1 \mu$  in length and being about one-half or two-thirds as thick. They sometimes appeared at temperatures lower than  $35^\circ \text{C}$ ., but less abundantly.

‡Fig. 137. Cross-section of a hyacinth-leaf showing xylem part of the bundle occupied by a bacterial cavity, parenchyma to either side being unoccupied. Leaf inoculated at apex in 1898 with a culture of *Bact. hyacinthi*. To either side of the bundle are (C C) natural passage-ways through leaf. Slide 502 B-A7.

show many long slender chains and also filaments 50 to 150  $\mu$  long in which no septa are visible (fig. 141). The organism is motile, and a polar flagellum has been demonstrated (fig. 142). Involution forms occur. Rods from young cultures stain readily; those from old cultures and from the overcrowded vessels take stains less freely. Wakker recommended Bismark brown (phenylene brown). The writer has used Ziehl's carbol fuchsin, and Gram's stain with amyl alcohol.

The purest color of the organism is bright yellow (gamboge, chrome, canary, or pale cadmium). The color is best developed in the plant and near the surface of fluid and solid culture media. When the air-supply is scanty the color is pale yellow. Duller and paler yellows occur also in certain media when oxygen is abundant, e. g. in potato-broths and acid beef-broths (not alkaline ones). In peptonized beef-bouillon, neutralized by sodium hydroxide, the color was canary yellow. The color was also bright in nutrient gelatin containing malic acid. This color appears to be a lipochrome compound, as it is associated with a fat. It is soluble in acetone, glycerin, a water solution of ammonium carbonate, or hydrogen peroxide, and slowly also in strong ammonia water, glacial acetic acid, ethyl acetate, ethyl alcohol, and methyl alcohol. The pigment can not be extracted with petroleum ether. The acetone extract, which also removes the fat, yields a blue-green or purplish reaction

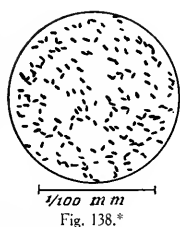


Fig. 138.\*



Fig. 139.†

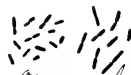


Fig. 140.‡



Fig. 141.§



Fig. 142.||

with concentrated sulphuric acid, and is readily destroyed by light. The yellow pigment is also bleached by reducing agents, the color returning on their removal (for further details consult Bulletin 28). The brown stain appears to be similar to that developed by *Bact. campestris*, but is less pronounced. It is soluble in water, and free oxygen appears to be necessary for its formation. It was best developed in hyacinth-broth, potato-broth with peptone, and on steamed turnips, radishes, and yellow banana rinds standing in distilled water. Sienna and burnt umber were the darkest shades observed (old cultures on radish and turnip). It was not observed in beef-broth, nutrient agar, starch-jelly or nutrient gelatin. It occurs in the plant, so far as observed, only in the vascular bundles of the leaves, and is not pronounced. In nutrient media it is best observed in old cultures.

*Bact. hyacinthi* is not sensitive to dry air. Wakker made this discovery and the writer has confirmed it. Thirteen cover slips, which were spread by the writer with a thin layer of bacteria from a young potato-culture and dried for 9 days, each infected culture media when thrown into it. Two of the same covers dried for 47 days at room-temperatures also yielded pure cultures when thrown into beef-bouillon. In another experiment 17 out of 18 similar covers infected beef-bouillon after being dry for 49 days (compare in this particular with *B. tracheiphilus* and *B. carotovorus*).

\*FIG. 138.—A detail of *Bact. hyacinthi* from fig. 137 at  $\times$ . Slide 502 B-A7.

†FIG. 139.—Rods of *Bact. hyacinthi*.  $\times 4000$ . After Wakker, Verslag, 1883, pl. I, fig. 1.

‡FIG. 140.—Rods of *Bact. hyacinthi*: a, directly from bulb; b, from a young beef-bouillon culture.  $\times 1000$ .

§FIG. 141.—Filaments of *Bacterium hyacinthi* from a culture on cane-sugar agar, segments not visible. Stained by van Ermengem's nitrate of silver method.  $\times 1000$ .

||FIG. 142.—Rods of *Bacterium hyacinthi*, showing flagellum: a, stained by V. A. Moore's modification of Loeffler's method; b, stained by Alfred Fischer's method.  $\times 1000$ .

This organism does not produce acids in milk, but there is a slowly increasing alkalinity, and after some days (3 to 10 or more) the casein is precipitated as a finely divided, voluminous, mobile mass, which settles slowly. These phenomena are best observed in litmus-milk. The litmus in such cultures is slowly reduced, but on the death of the organism it is oxydized back into a deep blue. In the end the casein is partially peptonized, but this change does not occur rapidly. The organism makes a good growth in milk and forms a bright yellow rim (plate 20, figs. 2-4), and sometimes a pellicle. In old cultures sheaf-like crystals of tyrosin occur.

In April, 1898, two 10 cc. tubes of milk, which had received 4 steamings and been under observation for a month unchanged, received 200 mgs. each of thymol. One was put away as a check, the other received 8 cc. of whey from a milk-culture of *Bact. hyacinthi* 33 days old, after this had been heated in the water bath for 10 minutes at 51.8° C. (4° above the thermal death point). There was no change in the check tube. In the other, there was copious precipitation of the casein in 48 hours, but no evidence of bacterial growth either then or subsequently (11 days). In October, 1898, the experiment was repeated with the same result. In this experiment (fig. 143) 10 cc. of sterile milk received 3 cc. of whey from

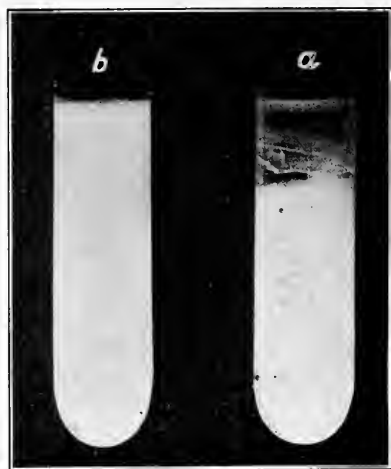


Fig. 143.\*

a milk-culture 10 days old. One hour after adding the whey the tube was heated for 20 minutes at 52° C. in the water bath to destroy the bacteria. In 24 hours the milk was entirely coagulated. A small drop from this tube was now transferred to bouillon but did not cloud it (5 days). At the same time another tube of the same milk received 3 cc. of whey from another milk-culture of the same organism, the only difference being that in this case the milk was heated for 10 minutes at 80° C. after adding the whey: This, to destroy the supposed enzyme. Result: No change in the milk (7 days). These experiments indicate the presence of a lab ferment.

In litmus-milk (and in bouillon) containing ethyl alcohol, a volatile acid is formed, and there is a fragrant odor in the steam. Methyl alcohol is not decomposed.

There is a moderate, smooth, wet-looking growth on steamed potatoes standing in distilled water, but it is not prolonged or copious. The color on potato is at first wax-yellow, but after some time it is dulled to a brownish yellow. At 20° C. to 25° C. the streak is not visible until the second or third day when inoculations are made from fluids. Growth on potato-cylinders is much increased by the addition of a little cane-sugar, dextrin, or malt-diastase. The action of the organism on starch is feeble, and the water surrounding the potato is never converted into a solid mass of slime as in case of *Bact. phaseoli*, *Bact. campestre*, *Bact. juglandis* and other starch-destroying organisms. On potato cylinders first soaked in pure water to remove the slight amount of sugar and acids on the cut surface and then tubed, growth did not occur or was delayed and scanty. Young cultures have no smell; in old cultures there is a feeble odor. Type of growth on potato like *Bact. stewarti* (Pl. 17, fig. 2).

\*Fig. 143.—Two tubes of sterile milk to which was added equal volumes of whey from old cultures of *Bact. hyacinthi* in milk. Whey added to tube b after heating to 80° C. (to destroy enzyme); whey added to tube a after heating to 52° C. (sufficient to destroy bacteria only). Result: Milk curdled promptly in tube a and remained unchanged in the other. Oct. 1898.

On yellow turnips prepared in the same way, growth was very much greater than on potato. Such turnips contained much more sugar than the potato. Turnip and carrot cylinders were softened by the long continued growth of this organism (middle lamellæ).

Growth on nutrient starch-jelly is also very slow, even when hyacinth-starch is used. When diastase was added to the jelly, increased growth was apparent at once (see Smith, Bulletin 26, plate I, figs. 15-16), and at the end of 35 days this was estimated at 200 times the volume in the check-tubes. At the end of 62 days (water being well retained by the medium) there was a thin canary-yellow layer over the surface of the check-tubes (Stock 310, for composition see *Bact. phaseoli*) equal to the growth given by the other tubes at the end of 5 days. The body of the starch in the check-tubes still preserved its bluish lustre, and on testing with Soxhlet's solution for sugar more than nine hundred and ninety-nine one thousandths of the starch was found unchanged. The only copper reduction on boiling 3 minutes was in an exceedingly thin film immediately under the bacterial layer. No brown pigment was formed on this substratum, with or without the diastase, and the color of the slime was much brighter yellow than that in corresponding tubes of *Bact. campestre* or *Bact. phaseoli*. There is always a strong iodine-starch-reaction, even in old cultures on starchy media, but some of the starch gives a red reaction (amylo-dextrin).

Gelatin (fig. 144) and Loeffler's blood serum are liquefied, but the change takes place slowly, does not occur in the absence of air, and is usually inhibited by the presence of 5 or 10 per cent grape-sugar or cane-sugar.

Dextrin stimulates growth; glycerin in small doses does not increase growth (?); in large doses it retards growth. In moderate doses grape-sugar, fruit-sugar, and cane-sugar stimulate growth. Lactose,

maltose (?) and mannitol have no marked effect on growth. *Bact. hyacinthi* made a very feeble growth in a synthetic medium made as follows: Distilled water 400; sodium acetate 2; dipotassium phosphate 0.8; magnesium sulphate 0.04; ammonium phosphate 0.04. The behavior was much the same as in Uschinsky's fluid.

This organism does not produce gas, and will not grow in the closed end of fermentation tubes in peptone-water, or peptonized beef-bouillon with grape-sugar, fruit-sugar, cane-sugar, milk-sugar, maltose (?), galactose, dextrin, glycerin, mannitol, ethyl alcohol, methyl alcohol, or potassium nitrate. For some days there was no growth in the closed end of the tubes containing peptonized beef-bouillon and ordinary commercial maltose, but in the end there was a *very feeble* clouding in the closed end. Two repetitions of the experiment gave the same result, and no air-bubble appeared in the closed end of the tube on subsequently steaming it. In a third repetition made in 1906 using a very pure maltose there was no growth in the closed end (fig. 145). The organism is therefore, as far as known, a

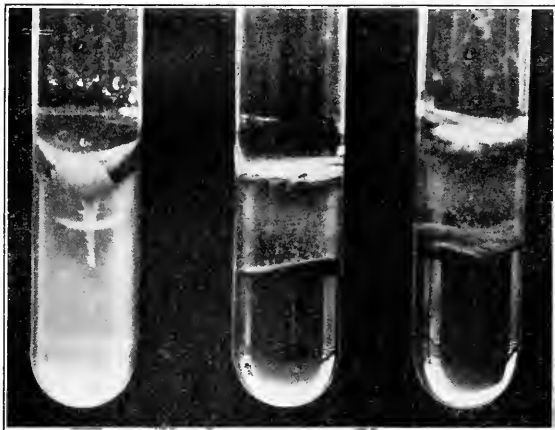


Fig. 144.\*

\*FIG. 144.—Old stab-cultures of *Bact. hyacinthi* in gelatin containing 0.6, 0.8 and 0.9 per cent malic acid showing slow liquefaction confined to upper part of gelatin. Rims and precipitate bright yellow.

strict aerobe, except in the presence of maltose or some unknown substance sometimes contaminating the maltose; a matter open to further inquiry. Cane-sugar is inverted. Oxydase and peroxydase are absent, *i. e.*, cultures give no reaction with guaiac resin in alcohol, nor on addition of hydrogen peroxide. Tyrosinase perhaps occurs, *i. e.*, the substance causing the brown stain. Catalase is present, *i. e.*, some body yielding a copious evolution of gas when hydrogen peroxide is added to old potato cultures. This substance is destroyed at 85° C.

Large doses of grape-sugar or cane-sugar in slant agar retard growth at first (9 per cent grape, 17 per cent cane) and then stimulate it greatly. On 10 grams of recently slanted nutrient agar containing 3 grams of grape-sugar no growth was obtained.

A small amount of non-volatile acid is developed out of various sugars—grape-sugar, fruit-sugar, cane-sugar, galactose, maltose; and in old cultures on the following substrata: carrot (occasionally), rutabaga, sweet potato, sugar-beet.

The steam from old cultures in hyacinth-broth caused a copious rusty precipitate when conducted into Nessler's solution, indicating the presence of ammonia or amines.

This organism grows readily on all ordinary culture-media except when it is too salt or too acid. It is very sensitive to acids, even those in the parenchyma-juice of the hyacinth retard growth (clouding) decidedly (14 days, 16 days, or more). Growth did not take place in potato-broth (+30), in juice of slow-growing cabbage leaves (+49), cauliflower-broth, sugar-beet juice diluted with water, or juice of green or ripe tomato-fruits (+59 and +64); growth was also much retarded in acid beef-broth (+40). The bacterium would not grow in beef-broth concentrated by boiling (+80). When the acidity of the +30 potato-broth was reduced slightly (+28, +26), by sodium hydrate, growth took place.

Growth in beef-broth was retarded (5 to 7 days) by 1.5 per cent c. p. sodium chloride.

It does not grow well in Uschinsky's solution; in this medium there was either no growth or it was long delayed and feeble (and without much yellow color) unless peptone was added to it, in which case growth was centupled.

Methylene blue in Dunham's solution was reduced within a few days, but reoxydized quickly on shaking, and was bright blue on the death of the organism, the bacterial precipitate being unstained. Indigo carmine in Dunham's

solution changed from a dull blue to a bright blue and retained this color for a long time, but finally became yellowish. Rosolic acid in Dunham's solution with enough c. p. hydrochloric acid to render the medium yellowish did not redden but became colorless; the bacterial precipitate, on the contrary, became rosy or salmon colored. Acid fuchsin in Dunham's solution bleached slowly, the color being all gone in about 4 weeks. Litmus in various media was bleached very slowly, the reduction being evident usually only at the close of the second or third week.

The optimum alkalinity for growth in peptonized beef-bouillon lies between 0 and +15 of Fuller's scale; the maximum tolerated alkalinity (sodium hydroxide in bouillon) is more than -20 and less than -40; in bouillon the tolerated acidity is about +30 (malic acid)

\*FIG. 145.—Behavior of *Bact. hyacinthi* in a fermentation-tube containing water, 1 per cent Witte's peptone, and 1 per cent maltose (the latter 3 times recrystallized). Culture cloudy in open end and clear in closed end after 8 days at 24° C. No gas. Drawn Dec. 18, 1906.

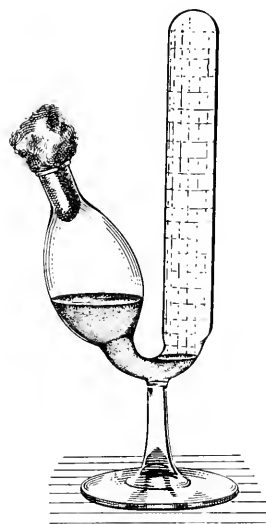


Fig. 145.\*



to +40 (lactic acid). Growth was retarded decidedly by +30 bouillon (to the 18th day or longer).

This organism produces indol in peptonized beef-broth or peptonized Uschinsky's solution, but not so abundantly as *Bacillus coli*. Lead acetate paper was browned, indicating slow evolution of hydrogen sulphide, when kept in the top of the test tube over certain cultures, *c. g.*, coconut-cylinders (fig. 146<sup>1</sup>), but not when kept over others, *c. g.*, potato-cylinders, turnip-cylinders (fig. 146<sup>2</sup>). In most cases, if the culture-medium developed the brown stain the sensitive paper remained unstained; if the culture remained free from the brown pigment the lead acetate paper was darkened. The only exception noted was yellow globe turnip: here both paper and substratum were browned.

Nitrites are not produced from organic nitrogen (beef-broth, peptone), nor from potassium nitrate in peptonized beef-broth.

This organism is not a strong smelling germ. It is not readily destroyed in ordinary culture-media by its own decomposition products nor in mixed growths.

It grows well with a bright yellow color and without retardation on steamed coconut-flesh, standing in distilled water. On this medium in a scanty air-supply (in vacuo, mercury at 3 inches) the growth was paler yellow than on the checks (bulk for bulk, examined on white paper); the same result was obtained on potato.

In agar and gelatin the growth is best toward the surface of the stabs. A whitish chemical halo forms slowly on the surface around the bacterial growth; this is soluble in acids, and does not appear when grape-sugar or cane-sugar is added. Growth does not occur in an atmosphere of pure hydrogen, nitrogen, or carbon dioxide, and exposure to these gases retards subsequent growth in the air, or prevents it altogether, if the exposure is longer than a few days. The organism is more tolerant of these gases on some media than on others, *c. g.*, subsequent growth in air after 10 days' exposure to carbon dioxide was retarded in beef-broth, but not on coconut cylinders. Growth in vacuo is feeble or altogether wanting, according to the completeness of the exhaustion of the air.

Buried colonies in agar and gelatin are small, grow slowly, and show no strong tendency to break through to the surface. Surface colonies on agar and gelatin are round, smooth, wet-shining, pale yellow with a thin distinct margin and are not rounded up much. They grow slowly (fig. 147, and Bull. 26, plate 1, fig. 12). Streaks on sugar agars sometimes developed the surface shown in fig. 148.

The best growth in gelatin was in that made on Fuller's scale.

The growth in gelatin slightly acid or slightly alkaline to litmus was not nearly so good; growth was good, however, in o gelatin to which a small amount of malic acid was subsequently added (+48 and +54 with 5 and

10 per cent cane sugar). Growth was very poor on acid and alkaline peptonized beef-broth gelatins (+40 and -20). Streak cultures came up slowly, even on the best (10 per cent)

\*FIG. 146.—Production of hydrogen sulphide by *Bact. hyacinthi*: 1, Lead acetate paper exposed (and browned) over culture on coconut flesh; 2, the same after exposure over cultures on potato or carrot.

†FIG. 147.—Strips of Petri-dish agar-poured plates of *Bact. hyacinthi*, showing slow growth of the colonies: *a* was photographed at end of 16 days at about 23° C.; *b* was poured Nov. 13, 1906, in +15 peptonized beef-bouillon, 1 per cent agar, incubated at 15° to 26° C., and photographed Dec. 25. Room temperatures.



Fig. 146.\*

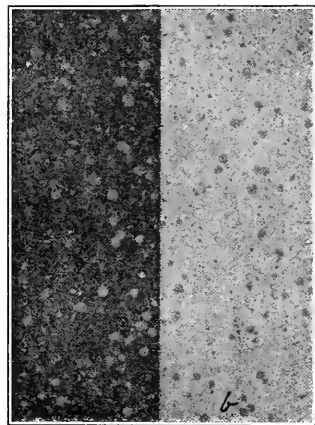


Fig. 147.†

gelatins. Cane-sugar added to the gelatin favors long continued growth, especially if the medium is quite alkaline on the start.

Minimum temperature for growth approximately  $4^{\circ}\text{C}.$ ; optimum temperature  $28^{\circ}\text{C}.$  to  $30^{\circ}\text{C}.$ ; maximum temperature approximately  $34^{\circ}$  to  $35^{\circ}\text{C}.$  *Bact. hyacinthi* will not grow in the thermostat at  $37^{\circ}\text{C}.$ , and grows very feebly on some media and not at all on others at  $34^{\circ}$  to  $35^{\circ}\text{C}.$  Thermal death point  $47.50^{\circ}\text{C}.$ , most of the rods are killed at  $46.50^{\circ}\text{C}.$

Good media for long continued growth are litmus-milk, sugar-beet cylinders in water, and nutrient gelatin neutralized to phenolphthalein with sodium hydrate and then acidified with malic acid (+50) and dosed with 5 per cent cane-sugar. The vitality on culture-media (except at high temperatures) usually varies from 3 or 4 weeks to as many months, 156 days and 174 days being the oldest viable cultures observed; these vigorous old cultures were on -30 gelatin, and on 0 gelatin with 10 per cent cane-sugar and malic acid to read +54 of Fuller's scale, the temperature ranging from  $10^{\circ}$  to  $25^{\circ}\text{C}.$

The slow development of the parasite in the host plant is attributed to its feeble action on cell-walls, its feeble action on starch, its sensitiveness to acids, and its strict aerobism.

#### RESUMÉ OF SALIENT CHARACTERS.

##### POSITIVE.

Pathogenic to *Hyacinthus orientalis* causing a yellow disease of the vascular system and finally a decay of the bulbs; a short motile rod, single, in pairs or 4's end to end, with rounded ends and one polar flagellum (chains and filaments in sugar-rich media); pseudo-zoogloecae and involution forms; stains readily from young cultures in basic anilin dyes; bright yellow in the host plant and on media; a slow grower; surface colonies flat, roundish, smooth, wet-shining; aerobic; inverts cane-sugar; slowly liquefies nutrient gelatin and Loeffler's solidified blood-serum; liquefaction of gelatin prevented by addition of cane-sugar in sufficient quantity; dissolves middle lamella in hyacinth, and softens it in turnip and carrot, but only very slowly; produces a non-volatile acid in small quantities from grape-sugar, fruit-sugar and cane-sugar, and a volatile acid and probably also an ester (steam fragrant) from ethyl alcohol; grows well in milk, forming a bright yellow rim and tyrosin crystals, leucin (?); blues litmus milk, precipitating the casein (by means of a lab ferment) as a mobile fluid

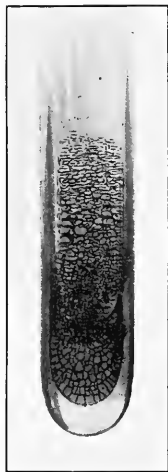


Fig. 148.\*

which settles slowly and becomes partially peptonized—tyrosin crystals appear slowly; growth on potato-cylinders not long continued nor very copious (iodine-starch-reaction always present, *i. e.*, diastatic action feeble); tolerates sodium hydrate to beyond -20 on Fuller's scale, also tolerates malic acid in beef-bouillon to about +30 and lactic acid to about +40; growth very slow on nutrient starch-jelly, much improved by addition of diastase; growth retarded by glycerin and by large doses of grape-sugar (9 per cent), or cane-sugar (17 per cent); streaks on the sugar-agars (9 to 23 per cent) were dry (not syrupy) and were variously areolated, reticulated, wrinkled or shagreened; growth feeble in Uscinsky's solution, better with peptone added; dextrin stimulates growth; sensitive to sodium chloride and to acids, *e. g.*, lactic, oxalic, acids of gelatin, growth retarded in hyacinth-juice and in other acid plant-juices; grows slowly and with much difficulty in bouillon over chloroform; moderate development of hydrogen sulphide; reduces litmus slowly; methylene blue in Dunham's solution reduced, final color bright blue; indigo-carmin in Dunham's solution becomes bright blue; rosolic acid in Dunham's solution becomes colorless and the bacterial precipitate is stained; acid fuchsin in Dunham's

\*FIG. 148.—Shagreen surface of *Bact. hyacinthi* on slant agar containing much cane-sugar.

solution is bleached slowly; resists drying; is not readily destroyed by its own decomposition products; forms indol sparingly; yellow pigment readily soluble in glycerin, acetone, and aqueous solution of ammonium carbonate; acetone extract destroyed by light and rendered blue-green or purplish by concentrated sulphuric acid; brown stain in hyacinth-broth, and on radish, turnip, etc.; slight pink stain (trace) in nutrient starch-jelly (made with peptone-water or Uchinsky's solution) with diastase; minimum temperature  $4^{\circ}\text{C}.$ , optimum  $28^{\circ}$  to  $30^{\circ}\text{C}.$ , maximum  $35^{\circ}\text{C}.$ , thermal death-point  $47.5^{\circ}\text{C}.$ —most of the rods in tubes of bouillon are killed by 10 minutes' exposure at  $46.5^{\circ}\text{C}.$ ; destroyed by sunlight. Group No. 211.2322523.

#### NEGATIVE.

Endospores; capsules (?); Gram's stain; gas; acids (from milk, glycerin (?), coconut flesh); assimilation of lactose, maltose, methyl alcohol; growth on agar streaks with 23 per cent grape-sugar; anaerobism (no growth in vacuo or gases, nor in closed end of fermentation tubes except with impure maltose); solubility of yellow pigment in petroleum ether; brown stain (agar, gelatin, peptonized beef-broth); strong odors; growth after exposure to sunlight 30 min., and 45 min. (thin sowings in agar plates); reduction of nitrates to nitrites; growth at  $37.5^{\circ}\text{C}.$ ; action on starch (nearly); growth in media decidedly acid to litmus; action on cell-walls of potato; Cohn's solution?

Any organism developing endospores readily, producing gas, or growing anaerobically with grape-sugar, or cane-sugar, having a strong diastasic action, *c. g.*, filling the water around potato cylinders in test tubes with a solid yellow slime, tolerating much acid, reddening litmus milk, liquefying gelatin quickly, producing a greenish stain, or forming a white growth on agar or potato, may be set down at once as something else.

#### TREATMENT.

In Holland the growers fight the disease by eradicating the diseased plants as fast as they are discovered. With these they commonly remove a little of the surrounding earth, but of course they can not remove all the infectious bacteria. From blossoming time on, the fields are searched regularly for the presence of this disease. The growers shade their eyes and the plants from the sun with bluish-green umbrellas, and this light enables them to see the disease on the parts above ground readily. This is the correct practice. Those plants which are found to be diseased in the field should be removed immediately and destroyed. They should never be allowed to decay in place, nor, when removed, should they be thrown into the canals, or upon the rubbish heaps. If conveniences are at hand, they may be burned, if not, they may be thrown into glass jars containing dilute, crude sulphuric acid to which more acid is frequently added. The bacterium is very quickly killed by acids, even when dilute. It goes without saying that diseased bulbs should never be planted. If diseased bulbs are used for the production of other bulbs either by notching or by the removal of the plateau, some of the daughter-bulbs are quite certain to contract the disease. If the disease is disseminated by insects, as seems not unlikely, means should be devised for their extermination. This subject deserves very careful inquiry.

By rubbing the yellow slime over the cut surface of bulbs Wakker secured infection of the vascular bundles of the youngest scales in 14 days, and in those of the older scales a little later. It appears probable, therefore, that the tools used by the gardeners sometimes transmit the disease. Growers may at least assume this to be true and govern themselves accordingly. Knives and other instruments used for cutting diseased bulbs should not be used on healthy bulbs until they have been disinfected, which may be done by soaking them for a short time in 5 per cent carbolic acid, 5 per cent lysol, 0.5 per cent formalin (strong) 0.1 per cent mercuric chloride, or in boiling water. A very short exposure to boiling water

is sufficient and this is perhaps the best method. An extra supply of knives will allow of sterilization without loss of time. For the same reason, in setting out bulbs the greatest care should be exercised not to wound them.

On May 20, 1883, Wakker removed the foliage from 17 bulbs when the leaves began to show signs of the disease. On September 26, only one of these bulbs was diseased. The other 16 were potted and bloomed the following April, and the bulbs were still sound when re-examined in June. This experiment was repeated by him several times with the same result. It was also tried by some of the Dutch growers with entirely confirmatory results. There can be no doubt, therefore, that early removal of infected leaves will preserve the bulb from infection. This shows very clearly that bulbs are often infected from the leaves. The writer believes that natural infection also takes place through the flower cluster and that insects will be found to be carriers of this disease.

A clue to the best method of eradicating the disease is afforded by the fact that these plants show a marked difference in susceptibility, some varieties contracting the disease readily and others being entirely or nearly immune, as shown in the remarks under *Étiology*.

If we can depend upon the statements respecting susceptibility there is good ground for thinking that many resistant varieties with other desirable qualities might be originated by cross-breeding and selection. The future of hyacinth growing on infected lands in Holland depends to a considerable extent, it would seem, on taking advantage of this fact. I can not think of any better means of eradicating this disease than by the origination of varieties which are not subject to it. This can probably be accomplished by using for one parent hyacinths which are not subject to this disease, and for the other those having other desirable qualities. From their progeny, for continued propagation, should be selected only those kinds which combine resistance to disease with other good qualities. In this way it is likely many resistant varieties of desirable character could be originated, but only at a very great outlay of time and trouble, since it requires about 7 years to grow bulbs from seed to a size suitable for market. The work of originating and fixing desirable strains would probably require several decades and were best done by the Government or by expert propagators, subsidized by the growers. Meanwhile diligent search should be made to know whether it is not possible to reduce the amount of the disease by the discovery and destruction of some insect carrier.

#### PECUNIARY LOSSES.

The writer knows nothing very definite as to the extent of the losses in the Netherlands. His only guide is the general statement of Dr. Wakker that it is one of the most serious diseases of the bulb in Holland, and statements made to him in Holland in 1906. The disease continues to be wide-spread and does much damage every year (p. 341). The majority of the fields are infected and there is no opportunity for shifting to new fields because there is only a small amount of good hyacinth land in Holland, and practically all of this is occupied.\*

#### HISTORY.

The disease has been known in Holland for a long time, but Wakker was the first to ascribe it to bacteria. For a full abstract of his papers see *American Naturalist*, 1896. The most important are in Dutch. No other Dutch writer has done much with the disease.

\*The best soil is a gray sand with a sandy subsoil and the soil-water within a foot of the surface. Heavier soils and soils with water at greater depths are not well adapted to the hyacinth.

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